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EFFECT OF THE AMOUNT AND DISTRIBUTION OF RAINFALL AND EVAPORATION DURING THE GROWING SEASON ON YIELDS OF CORN AND SPRING WHEAT [1]

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INTRODUCTION

The relation of weather to crop yields has been the subject of a large number of investigations in this country and abroad (*4*).[3] In general, the usual correlation method of approach, which employs State yields and monthly weather data, has failed to provide a reliable regression equation for forecasting purposes, although instances may be cited in which it has yielded valuable information. These studies serve to emphasize the complexity of the relationships involved.

Yields of most crops are dependent to a large extent upon the weather during the growing season. In the present work it was desired to ascertain the influence on yield of certain weather factors at different stages during the season, and to determine the critical period of the growing season as regards these factors.

In studying the effect of rainfall on plot yields of winter wheat, Fisher (*1*) conceived the idea that rainfall occurring at any time during the growing season has an effect on final yield which can be expressed as $a(t)r(t)dt$, where $r(t)dt$ is the rainfall occurring during the interval t to $t+dt$, and $a(t)$ is a function of time expressing the physiological relation between amount of rainfall at time t and final yield. On this basis, the estimated yield, the rainfall during the season being known, will be

$$\bar{w}=c+\int_0^T a(t)r(t)dt.$$

This expression simply indicates that the estimate of yield is the product (rainfall)$\times$(the yield functions $a(t)$) summed over the entire season. It is clear that $a(t)$ will in general be a continuous function and one that changes relatively slowly. It may be expected, therefore, that it can be approximated with reasonable accuracy with a few terms of a polynomial in t. As a device for evaluating $a(t)$, Fisher used a set of orthogonal polynomials.

As pointed out by Fisher, this approach assumes that the effect of rain at any time is independent of the amount of rain falling at any other time. Nevertheless, relationships learned in this manner can be of considerable value in an understanding of the influence of weather factors on crop yields at different stages during the season.

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[3] Italic numbers in parentheses refer to Literature Cited, p. 22.

Such knowledge is of value to crop forecasters even though the deviations from regression due to other factors may be large.

The primary purpose of this paper is to show the results of applying Fisher's technique to a study of weather factors and experiment station yields of corn and spring wheat. In view of the complicated nature of this technique, however, the paper is also designed to give the successive stages in the handling of the data. Improved methods of handling the computational work are also set forth. For the general method and theory one can profitably refer to Fisher's original paper, particularly to section 3.

Data regarding annual plot yields for a few crops were made available to the Bureau of Agricultural Economics by a number of agricultural experiment stations throughout the country so that relationships between weather factors and yields might be studied. The method used in this paper is one of several that are being employed in these studies. The selection of the particular crops and of the yield series presented in this report was made on the basis of the opportunity they afforded to introduce the use of the present technique to crops and to weather factors not previously studied and to bring out certain refinements in the techniques. One of the first considerations in relating weather to yields is that a sufficiently long series of records be available. Fisher's analysis dealt with 60 years of yields, but nowhere in this country is there any series of continuous plot yields of that length. However, many series are of sufficient length to justify analysis by this technique.

DATA USED

Corn (*zea mays*) yields from plots at the Ohio Agricultural Experiment Station at Wooster, Ohio, and spring wheat (*Triticum aestivum*) yields from the Dry Land Experiment Station at Dickinson, N. Dak., were used in this study.

Since 1894 at Wooster a series of plots has been cropped continuously to corn, with the more important weather factors observed concurrently. This series includes several treatments. The plots are located on Wooster silt loam and are slightly rolling. Plot 5, which has received a moderate treatment of manure each year (2½ tons per acre), has been used in this study. The use of lime on these soils was begun in 1905, and the yields from the date the liming practice was started have been used, giving a 32-year period (1905-36) with which to work.

The yield data for spring wheat were obtained from the original records of the Division of Dry Land Agriculture, Bureau of Plant Industry, United States Department of Agriculture.[4] Two plots receiving no fertilizer treatment have been cropped to wheat continuously since 1908. The spring wheat yields used are the averages of these two plots. (In 1912 hail destroyed the crop; consequently, no records for that year are included in the calculations.)

The meteorological data were obtained from the station records. Those used in this report are daily rainfall figures for the season at Wooster and daily rainfall and evaporation figures for the season at Dickinson. Before this study was begun these records had been drawn off and compiled into 5-day totals throughout the year.

[4] These records were obtained through the courtesy of J. S. Cole.

Since the winter and spring rainfall is nearly always adequate at Wooster, the decision was made to investigate the effect of rainfall for a period of 120 days beginning a few days before planting time. The average date of planting was found to be about May 13. The approximate date of planting was ascertained for each year and the 5-day period in which this date occurred was marked on the rainfall tabulation. Then the third 5-day period previous to this was marked and was considered the beginning of the 120-day season for that year. Thus, the season of 120 days was different for each year, depending on the date of planting. Since the rainfall for the 120 days is compiled into totals of 5 days each, 24 such totals represent the season.

For spring wheat at Dickinson, the average date of seeding is about April 16 and the average date of harvesting August 5, with a mean interval of around 111 days. Here, as in the case of corn, a period of 120 days would seem adequate, and since evaporation data were available from April 1 of each year, it was decided to consider the crop season as beginning on that date and continuing for 120 days.

ANALYSIS OF DATA

METHOD OF ANALYSIS

In a direct attack on the relationship of a large number of independent weather variates (twenty-four 5-day totals of rainfall for the season) with yield, one would ordinarily set up equations in 24 unknowns α (1), α (2), . . . α (24). The solution for these 24 regression coefficients directly would involve a tremendous amount of arithmetical work. Furthermore, with data for only 20 to 40 years, the high proportion of independent variables to observations would make it difficult to establish the significance of the regression coefficients. On physiological grounds it appears valid to assume that a coefficient of regression of yield on rainfall in one 5-day period would not differ widely from one in adjacent 5-day periods. Dropping the concept of a particular regression coefficient for a particular 5-day period, we can conceive a relation between yield and additional rainfall that changes slowly as we go from one short period of time to another. Such a function changing continuously with time is precisely what is to be represented by the function $a(t)$. Looking upon $a(t)$ as a slowly changing function we assume that it can be represented by a polynomial and hence by a set of orthogonal polynomials.

The problem is now to evaluate the function a in equation

$$\overline{W}=C+\int_0^\tau ardt \tag{1}$$

where a, as a continuous function, can be adequately represented by relatively few terms of a polynomial of the form

$$a=\alpha_0T_0+\alpha_1T_1+\alpha_2T_2+\ldots \tag{2}$$

the T's being the orthogonal functions of time. If (2) is substituted in (1) we get

$$\overline{W}=C+\int_0^\tau(\alpha_0T_0+\alpha_1T_1+\ldots)rdt$$

$$=C+\alpha_0\int_0^\tau T_0rdt+\alpha_1\int_0^\tau T_1rdt+\ldots \tag{3}$$

Now the quantities $\int_0^T T_t r dt$ are those one would get if orthogonal polynomials were fitted to the sequence of rainfall each year. From actual rainfall data in those years for which crop yields are available, one can calculate the quantities $\int_0^T T_t r dt$. Placing these quantities in equation (3), the coefficients α_0, α_1, . . . can be computed by multiple regression methods. Substituting the calculated partial regression coefficients in equation (2), we can evaluate the function a and represent at any time during the season the effect on the crop of an additional inch of rainfall.

In the application of his scheme, Fisher expressed a polynomial fitted to the sequence of rainfall in each year, as mentioned above, by

$$\rho_0 T_0 + \rho_1 T_1 \ldots \tag{4}$$

in which ρ_t represents the quantities $\int_0^T T_t r dt$ and the T's are orthogonal and normalized functions of the time, that is,

$$\int_0^T T_r T_s dt = 0 \quad (r \neq s)$$

$$\int_0^T T_r^2 dt = 1$$

The values ρ_t may be calculated thus:

$$\rho_t = S(rT_t)$$

where r equals rainfall during a 5-day period and the summation extends over the given number of periods, noting that the integrations have been replaced by the summations.

But the numerical values are awkward to handle, and it is simpler arithmetically to use polynomials ξ_t' which are orthogonal but not normalized, and the values of which are integers. The polynomials are those tabulated by Fisher and Yates (*3*) and are related to those of Fisher by

$$\xi_t' = T_t \sqrt{S(\xi_t'^2)}$$

Employing the polynomials ξ_t' instead of the polynomials T_t, a set of coefficients ρ_t is calculated that are related to the coefficients used by Fisher. The process of calculations

$$\rho_t' = S(r\xi_t')$$

is similar to that given above for ρ_t. The amount and distribution of rainfall is now expressed as a function of time by

$$\frac{\rho_0' \xi_0'}{S(\xi_0'^2)} + \frac{\rho_1' \xi_1'}{S(\xi_1'^2)} + \ldots \tag{5}$$

The quantities here are related to those in Fisher's expression (equation (4)) by the equations

$$\xi_t' = T_t\sqrt{S(\xi_t'^2)}$$

$$\rho_t' = \rho_t\sqrt{S(\xi_t'^2)}$$

Since the values $S(\xi_t'^2)$ are given in the tables, Fisher's coefficients ρ_t could easily be calculated, but it is to be shown here that these numerous divisions may be avoided.

If the regression of yield on the coefficients $\rho_0', \rho_1', \rho_2', \ldots$ is calculated, the partial regression coefficients obtained may be designated as $\alpha_0', \alpha_1', \alpha_2', \ldots$. These regression coefficients are related to the corresponding values $\alpha_0, \alpha_1, \ldots$ obtained by Fisher, in this manner:

$$\alpha_t' = \frac{\alpha_t}{\sqrt{S(\xi_t'^2)}}$$

To obtain values for the regression function a, Fisher uses

$$a = \alpha_0 T_0 + \alpha_1 T_1 + \ldots$$

In terms of the polynomials $\xi_0', \xi_1', \ldots$ and the writers' coefficients α_0', α_1', etc., this becomes

$$a = \alpha_0'\xi_0' + \alpha_1'\xi_1' + \ldots \tag{6}$$

by virtue of the relations previously stated and summarized as follows:

$$\alpha_t'\xi_t' = \frac{\alpha_t}{\sqrt{S(\xi_t'^2)}} T_t\sqrt{S(\xi_t'^2)} = \alpha_t T_t$$

This means that the orthogonal polynomials tabulated by Fisher and Yates (*3*) are convenient not only for fitting polynomials but also in the expansion of the regression integral and subsequent evaluation of the function a.

ANALYSIS OF THE CORN DATA

From the rainfall in each of the twenty-four 5-day periods of each season designated by $r_1 \ldots r_{24}$, the values of $\rho_0', \rho_1', \ldots \rho_5'$ as tabulated in table 1 were calculated by the use of the ξ''s mentioned above, where

$$\rho_0' = S(r\xi_0') \text{ summed over the 24 periods}$$
$$\rho_1' = S(r\xi_1') \text{ summed over the 24 periods}$$
$$\vdots$$
$$\rho_5' = S(r\xi_5') \text{ summed over the 24 periods}$$

using the tabular values of ξ''s for n equals 24. As ξ_0' is a constant, 1, the value ρ_0' will be the sums of $r_1 \ldots r_{24}$ which is the total rainfall for the season of 120 days.

As a curve of the fifth degree was being fitted it was worth while to actually work from the center of the 24 values and to build up a column of sums and differences of paired values around the center. The differences were then used with ξ_1', ξ_3', and ξ_5' while the sums were used with ξ_2' and ξ_4' (see Fisher and Yates (*3*)).

The object is now to secure the regression of yield on the six series $\rho_0' \ldots \rho_5'$. Before doing this it was advisable to look at the yields over the period to see if any slow changes had taken place. As expected on such continuous plots, the tendency has been for the level of yields to decline gradually. A linear regression was used to remove the slow changes since examination showed that it accounted for a major portion of the variation. It is recognized that in other problems slow changes may be expressed better by a parabola or some polynomial of higher degree.

TABLE 1.—*Corn yields from continuous plot 5 at Wooster, Ohio, 1905–36, seasonal rainfall distribution coefficients, and values of* ξ_1' *for* $n=32$

Year	Yield per acre	ρ_0'	ρ_1'	ρ_2'	ρ_3'	ρ_4'	ρ_5'	ξ_1'
	Bushels							
1905	45.7	22.47	18.75	−738.4	4,507	765	10,705	31
1906	34.3	17.10	92.30	239.2	902	−98	3,481	29
1907	17.7	13.96	−24.96	−263.5	416	−88	−5,147	27
1908	20.8	13.99	−4.07	262.4	−2,252	−437	−1,258	25
1909	19.4	19.73	−3.89	213.0	2,403	496	9,202	23
1910	12.8	9.55	−77.23	82.0	1,896	−280	−3,892	21
1911	32.0	11.62	6.86	−364.8	743	318	2,926	19
1912	31.6	20.32	28.82	306.3	−3,824	−331	2,150	17
1913	20.0	12.17	45.03	317.2	2,184	199	7,406	15
1914	22.9	14.69	−6.29	99.8	2,217	368	−5,661	13
1915	32.9	21.50	68.68	−844.6	−2,352	6	−3,452	11
1916	19.0	12.83	−38.03	−404.8	1,167	182	−11,634	9
1917	24.2	13.26	−21.70	−258.3	3,550	226	−4,142	7
1918	16.9	11.57	−42.05	151.2	1,061	−454	−2,918	5
1919	33.1	21.36	40.02	420.7	−6,094	−732	13,180	3
1920	22.3	19.24	52.40	−443.2	4,926	496	−3,142	1
1921	20.1	12.44	−33.26	−115.2	−1,540	153	−6,448	−1
1922	9.6	14.49	−72.85	−412.0	1,044	−192	16,069	−3
1923	20.3	12.82	3.74	603.8	−684	−464	12,692	−5
1924	14.1	17.38	−49.88	−220.8	628	40	472	−7
1925	27.3	11.12	16.16	−44.0	−3,864	25	3,377	−9
1926	29.8	11.00	12.86	−105.2	1,606	356	4,317	−11
1927	21.1	15.06	−18.74	−22.4	−2,142	−523	2,238	−13
1928	12.1	14.55	43.27	−499.1	528	−196	−7,442	−15
1929	9.9	15.76	−55.00	−772.0	−1,852	700	4,762	−17
1930	15.0	6.91	.07	−352.0	−734	102	1,830	−19
1931	34.1	15.25	10.89	364.5	3,353	−117	11,998	−21
1932	23.7	10.52	4.08	−171.0	−1,852	421	6,670	−23
1933	6.9	12.01	−40.71	471.7	−3,335	−288	−5,966	−25
1934	25.6	11.77	103.85	46.4	841	584	2,968	−27
1935	29.0	27.89	95.45	−157.9	−14,772	−72	−20,027	−29
1936	21.1	12.10	30.02	−135.3	−2,733	−375	5,463	−31
Sum	725.3	476.43	184.59	−2,746.3	−14,058	790	40,777	0
Mean	22.67	14.89	5.77	−85.8	−439.3	24.7	1,274.3	

Although it was not expected that there would be any marked sign of change in the six series of values $\rho_0' \ldots \rho_5'$ they were examined. No changes were evident. As a linear regression is being fitted to the yields, it is also fitted to the series $\rho_0' \ldots \rho_5'$ in order not to disturb the tests of significance of the regression coefficients.

As the individual deviations of yield from the regression line are not needed elsewhere, the sums of squares and of products with $\rho_0' \ldots \rho_5'$ were obtained without calculating the six series of individual deviations from trend. The ξ' values of the first order for n equals 32 were employed in the following way:

$$S(y-E_{y\xi 1}'^{*})^2=S(y^2)-(Sy)^2/n-(S\xi_1'y)^2/S(\xi_1'^2)$$

and

$$S(y-E_{y\xi 1}')(\rho_1'-E_{\rho 0'\,0\xi 1}')=S(y\rho_0')-(Sy)(S\rho_0')/n-(\xi_1'y)(\xi_1'\rho_0')/S(\xi_1'^2)$$

* $E_{y\xi 1}'$ is the estimated value from the regression of y on ξ_1'.

because the sum of the squared deviations from linear regression

$$=S(y^2)-(Sy)^2/n-b^2_{y\xi 1}{}'\,S(\xi_1{}'^2)$$
$$=S(y^2)-(Sy)^2/n-\frac{(S\xi_1{}'y)^2}{[S(\xi_1{}'^2)]^2}S(\xi_1{}'^2)$$
$$=S(y^2)-(Sy)^2/n-(S\xi_1{}'y)^2/S(\xi_1{}'^2)$$

The computation of the sums of squares and products of deviations from linear trend line by the above expression from values in table 2 are illustrated below. The value 10,912 for $S(\xi,{}'^2)$ is obtained directly from the foot of the tables of ξ's for n equals 32 in the statistical tables compiled by Fisher and Yates (*3*).

TABLE 2.—*Sums of squares and of products of yield ρ_1' and ξ_1' from table 1*

Variable	Y	ρ_0'	ρ_1'	ρ_2'	ρ_3'	ρ_4'	ρ_5'	ξ_1'
y	18,774.35	11,350.83	11,774.50	−63,574.14	−346,358.4	35,069.5	1,360,207.8	1,423.9
ρ_0'		7,703.88	5,460.0	−48,195	−394,631	12,781	494,234	459.03
ρ_1'			68,364	12,876	−1,498,948	60,293	−334,207	−2,775.33
ρ_2'				4,479,341	−4,286,861	−2,342,311	15,563,474	2,304.5
ρ_3'					425,477,142	17,538,629	269,832,155	853,872
ρ_4'						4,553,478	895,211	12,742
ρ_5'							1,952,539,937	227,561
ξ_1'								10,912

For sums of squares:

$$S(y-E_{y\xi 1}{}')^2=18{,}774.35-\frac{(725.3)^2}{32}-\frac{(1{,}423.9)^2}{10{,}912}=2{,}149.17$$

and for sums of products:

$$S(y-E_{y\xi_1}{}')(\rho_0{}'-E_{\rho 0}{}'_{\xi_1}{}')=11{,}350.834-\frac{(725.3)(476.43)}{32}$$
$$-\frac{(1{,}423.9)(459.03)}{10{,}912}=492.352$$

These and the remaining values of the sums of cross products of yield with the rainfall distribution coefficients ρ_0' . . . ρ_5' with linear trend removed from each are:

$S(y\rho_0')=$ 492.35
$S(y\rho_1')=$ 7,952.80
$S(y\rho_2')=-1{,}628.25$
$S(y\rho_3')=-139{,}146.2$
$S(y\rho_4')=$ 15,501.0
$S(y\rho_5')=$ 406,227.3

Here and in succeeding pages relating to corn the notations y and ρ_1' refer to values with trend eliminated. The values of the sums of squares and products of deviations from linear trends fitted to the six series of rainfall distribution coefficients are shown in table 3.

TABLE 3.— *Wooster, Ohio, rainfall distribution coefficients: Sums of squares and products corrected for contributions of linear trend*

DIRECT

Variable	ρ_0'	ρ_1'	ρ_2'	ρ_3'	ρ_4'	ρ_5'
ρ_0'	591.28	2,828.4	−7,404	−221,248	483	−122,445
ρ_1'	2,828.4	66,594	29,304	−1,200,683	58,977	−511,549
ρ_2'	−7,404	29,304	4,243,161	−5,673,674	−2,277,203	19,014,974
ρ_3'	−221,248	−1,200,683	−5,673,674	352,485,177	16,888,615	269,939,185
ρ_4'	483	58,977	−2,277,203	16,888,615	4,519,096	−377,195
ρ_5'	−122,445	−511,549	19,014,974	269,939,185	−377,195	1,895,832,968

TABLE 3.—*Wooster, Ohio, rainfall distribution coefficients: Sums of squares and products corrected for contributions of linear trend*—Continued

IN CODE

Variable	$\rho_0'/_{10^1}$	$\rho_1'/_{10^2}$	$\rho_2'/_{10^3}$	$\rho_3'/_{10^4}$	$\rho_4'/_{10^3}$	$\rho_5'/_{10^4}$
$\rho_0'/_{10^1}$	5.9128	2.8284	−0.7404	−2.2125	0.0483	−1.2244
$\rho_1'/_{10^2}$	2.8284	6.6594	.2930	−1.2007	.5898	−.5115
$\rho_2'/_{10^3}$	−.7404	.2930	4.2432	−.5674	−2.2772	1.9015
$\rho_3'/_{10^4}$	−2.2125	−1.2007	−.5674	3.5248	1.6889	2.6994
$\rho_4'/_{10^3}$	.0483	.5898	−2.2772	1.6889	4.5191	−.0377
$\rho_5'/_{10^4}$	−1.2244	−.5115	1.9015	2.6994	−.0377	18.9583

The partial regression coefficients α_0', α_1', . . . α_5' of yield on rainfall distribution coefficients ρ_0' . . . ρ_5' may be obtained from the solution of the six simultaneous equations:

$$\begin{aligned}
&\alpha_0'S(\rho_0'^2) + \alpha_1'S(\rho_0'\rho_1') + \alpha_2'S(\rho_0'\rho_2') + \ldots + \alpha_5'(\rho_0'\rho_5') = S(y\rho_0') \\
&\vdots \\
&\alpha_0'S(\rho_0'\rho_5') + \alpha_1'S(\rho_1'\rho_5') + \alpha_2'S(\rho_2'\rho_5') + \ldots + \alpha_5'(\rho_5'^2) = S(y\rho_5') \qquad (7)
\end{aligned}$$

As the rainfall distribution coefficients may eventually be correlated with yield data from more than one plot, it was advantageous to calculate at this stage the values of the multipliers C_{rs}. (See *2*, *sec. 29*, for a discussion of C values.)

The solution for these C values appears in table 4 from the six sets of six simultaneous equations in the following manner:

$$\begin{aligned}
A_0.\ & C_{r0}S(\rho_0'^2) + C_{r1}S(\rho_0'\rho_1') + C_{r2}S(\rho_0'\rho_2') + C_{r3}S(\rho_0'\rho_3') \\
&\quad + C_{r4}S(\rho_0'\rho_4') + C_{r5}S(\rho_0'\rho_5') = 0,0,0,0,0,1 \\
A_1.\ & C_{r0}S(\rho_1'\rho_0') + C_{r1}S(\rho_1'^2) + C_{r2}S(\rho_1'\rho_2') + C_{r3}S(\rho_1'\rho_3') \\
&\quad + C_{r4}S(\rho_1'\rho_4') + C_{r5}S(\rho_1'\rho_5') = 0,0,0,0,1,0 \\
A_2.\ & C_{r0}S(\rho_2'\rho_0') + C_{r1}S(\rho_2'\rho_1') + C_{r2}S(\rho_2'^2) + C_{r3}S(\rho_2'\rho_3') \\
&\quad + C_{r4}S(\rho_2'\rho_4') + C_{r5}S(\rho_2'\rho_5') = 0,0,0,1,0,0 \\
A_3.\ & C_{r0}S(\rho_3'\rho_0') + C_{r1}S(\rho_3'\rho_1') + C_{r2}S(\rho_3'\rho_2') + C_{r3}S(\rho_3'^2) \\
&\quad + C_{r4}S(\rho_3'\rho_4') + C_{r5}S(\rho_3'\rho_5') = 0,0,1,0,0,0 \\
A_4.\ & C_{r0}S(\rho_4'\rho_0') + C_{r1}S(\rho_4'\rho_1') + C_{r2}S(\rho_4'\rho_2') + C_{r3}S(\rho_4'\rho_3') \\
&\quad + C_{r4}S(\rho_4'^2) + C_{r5}S(\rho_4'\rho_5') = 0,1,0,0,0,0 \\
A_5.\ & C_{r0}S(\rho_5'\rho_0') + C_{r1}S(\rho_5'\rho_1') + C_{r2}S(\rho_5'\rho_2') + C_{r3}S(\rho_5'\rho_3') \\
&\quad + C_{r4}S(\rho_5'\rho_4') + C_{r5}S(\rho_5'^2) = 1,0,0,0,0,0
\end{aligned}$$

In the first set of equations to be solved, equation A_5 is set equal to 1 and the others are set equal to 0. In succeeding sets a similar procedure is followed with a different equation being set equal to 1. The subscript r for the C values in any set of the equations follows that of the equation that is set equal to 1. The scheme of the solution followed in table 4 is that set out by Waugh (*8*).

The C values can be obtained in terms of the reduced equations in table 4. For the first set of six simultaneous equations these are obtained as follows:

$$C_{55} = 1/a_{55}' = 0.06557213$$
$$C_{45} = a_{45}''C_{55} = 0.00835061$$
$$C_{05} = a_{05}''C_{55} + a_{04}''C_{54} + a_{03}''C_{53} + a_{02}''C_{52} + a_{01}''C_{51} = -0.01840803$$

TABLE 4.—*Reduction of the simultaneous equations* $A_0 \ldots A_5$

Equation	Line	Reciprocal	0	1	2	3	4	5	Check
A_0	$a_{0'}$	−0.16912461	5.9128	2.8284	−0.7404	−2.2125	0.0483	−1.2244	4.6122
	$a_{0''}$		5.9128	2.8284	−.7404	−2.2125	.0483	−1.2244	4.6122
	$a_{0'''}$		−1.000000	−.178352	.125220	.371188	−.008169	−.207076	−.780037
A_1	$a_{1'}$	−.18845065		6.6594	.2930	−1.2007	.5898	−.5115	8.6584
	$a_{1''}$			5.306429	.647172	−.142346	.566696	−.074194	6.462145
	$a_{1'''}$			−1.000000	−.121960	.026825	−.106794	−.013982	−1.215911
A_2	$a_{2'}$	−.24560622			4.2432	−.5674	−2.2772	1.9015	2.8527
	$a_{2''}$				4.071558	−.827080	−2.340266	1.739132	2.643336
	$a_{2'''}$				−1.000000	.203138	.574784	−.427142	−.649220
A_3	$a_{3'}$	−.39602737				3.5248	1.6889	2.6904	3.9225
	$a_{3''}$					2.525078	1.246778	2.596518	6.368371
	$a_{3'''}$					−1.000000	−.493758	−1.026292	−2.522049
A_4	$a_{4'}$	−.40041130					4.5191	−.0377	4.5312
	$a_{4''}$						2.497432	−.318048	2.179396
	$a_{4'''}$						−1.000000	−.127350	−.872651
A_5	$a_{5'}$	−.05557213						18.9583	21.7856
	$a_{5''}$							15.250381	15.250382
	$a_{5'''}$							−1.000000	−1.000000

The values C_{r4}, C_{r3}, etc., are calculated by a similar process and the final figure to be calculated is:

$$C_{00}=1/a_{00}'+a_{05}''C_{05}+a_{04}''C_{04}+a_{03}''C_{03}+a_{02}''C_{02}+a_{01}''C_{01}=0.28956313$$

The $1/a_{rr}'$ are to be obtained in the solution in table 4 and entered (with sign changed) to the left of the a_{rr} values.

For convenience, a coding process was used on the sums of squares and products in table 3 as four or five significant figures were thought adequate. The coding was performed by divisions of powers of 10 by setting the decimal to the left the appropriate number of places. By inspection of the sums of squares, a divisor of a power of 10 best suited for each coefficient was selected. Remembering that exponents are added when multiplying numbers, the power of 10 is doubled in case of a sum of squares, and the exponents are added in case of a sum of products. Thus, for ρ_2' the divisor is 10^3 which becomes 10^6 on squaring and the sum of squares 4,243,162.10 is coded to 4.2432; and for the product of $\rho_1'\rho_2'$ the divisors are 10^2 and 10^3 which on multiplying become 10^5 and the sum of products of 29,303.646 is coded to 0.2930. The lower part of table 3 contains the coded values. Upon completion of the solution in table 5 it is

TABLE 5.—*Values of C_{rs}, in code*

r \ s	$C_{r0}.10^1$	$C_{r1}.10^2$	$C_{r2}.10^3$	$C_{r3}.10^4$	$C_{r4}.10^5$	$C_{r5}.10^4$
$C_{0s}.10^1$	0.289563	−0.092118	0.078552	0.187531	−0.021728	−0.018408
$C_{1s}.10^2$	−.092118	.204791	−.064093	.035030	−.071125	.000875
$C_{2s}.10^3$	.078552	−.064093	.373820	.027825	.185182	−.037743
$C_{3s}.10^4$	.187531	.035030	.027825	.571720	−.206818	−.071550
$C_{4s}.10^3$	−.021728	−.071125	.185182	−.206818	.401475	.008351
$C_{5s}.10^4$	−.018408	.000875	−.037743	−.071550	.008351	.065572

necessary to decode. Owing to the nature of the C values, the decoding is done by dividing again by the same powers of 10 rather than by multiplying as might be expected. The decoded C values appear in table 6.

TABLE 6.—*Values of C_{rs}, decoded*

r \ s	C_{r0}	C_{r1}	C_{r2}	C_{r3}	C_{r4}	C_{r5}
C_{0s}	−0.00289563	−0.0^{2}92118	0.0^{3}78552	0.0^{4}187531	−0.0^{3}21728	−0.0^{4}18408
C_{1s}	−.0^{2}92118	.0^{2}04791	−.0^{3}64093	.0^{3}35030	−.0^{2}71125	.0^{3}875
C_{2s}	.0^{3}78552	−.0^{3}64093	.0^{3}73820	.0^{3}27825	.0^{3}185182	−.0^{3}37743
C_{3s}	.0^{4}187531	.0^{3}35030	.0^{3}27825	.0^{3}571720	−.0^{3}206818	−.0^{3}71550
C_{4s}	−.0^{3}21728	−.0^{2}71125	.0^{3}185182	−.0^{3}206818	.0^{3}401475	.0^{3}8351
C_{5s}	−.0^{4}18408	.0^{3}875	−.0^{3}37743	−.0^{3}71550	.0^{3}8351	.0^{3}65572

The partial regression coefficient α_s' of any dependent variate y on ρ_s' may then be rapidly obtained from the equation:

$$\alpha_s'=C_{0s}S(\rho_0'y)+C_{1s}S(\rho_1'y)+C_{2s}S(\rho_2'y)+C_{3s}S(\rho_3'y)+C_{4s}S(\rho_4'y)+C_{5s}S(\rho_5'y) \quad (8)$$

The six regression coefficients are:

$$\alpha_0' = 0.310872 \qquad \alpha_3' = -0.000211$$
$$\alpha_1' = 0.103012 \qquad \alpha_4' = 0.002412$$
$$\alpha_2' = -0.000888 \qquad \alpha_5' = 0.00030138$$

These regression coefficients enable us in the present case to estimate the average effect on the crop of an extra inch of rainfall at any time within the 120-day period. Figure 1 shows the course of the effect

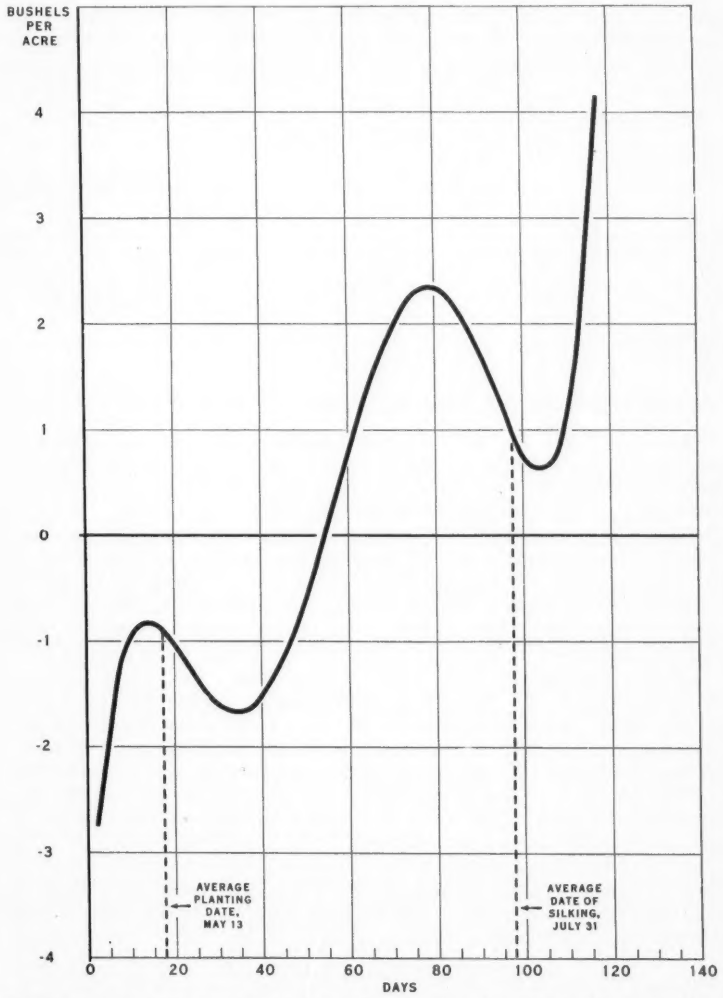

FIGURE 1.—Average effect in bushels per acre of each additional inch of rainfall on the yield of corn on continuously cropped plot No. 5 at Wooster, Ohio.

throughout the 120-day period. This is a continuous curve smoothed from the 24 points found by substituting successively the values of $\xi_0' \ldots \xi_5'$ for -23 to $+23$ in equation (6), $a = \alpha_0' \xi_0' + \alpha_1' \xi_1' + \ldots$, and using the above calculated values of $\alpha_0' \ldots \alpha_5'$.

The significance of the amount of variation in the annual yields accounted for by the regression formula may be investigated by determining the distribution of the total variance of yield, corresponding to 30 degrees of freedom (1 having been used in eliminating trend) between the 6 degrees of freedom of the regression formula and the 24 degrees of freedom appropriate to deviations of yield from the regression formula. From table 7 it is apparent that the regression formula has accounted for 54 percent of the total variance of yield. The ratio of the two mean squares is $F = 4.7$(6), a highly significant value.

TABLE 7.—*Analysis of variance of yield of corn*

Source	Degrees of freedom	Sums of squares	Mean square
Precipitation regression	6	1,162.93	**193.82
Deviation from regression	24	986.24	41.09
Total	30	2,149.17	
	$R^2 = 0.5411$ $R = .73+$		

**Highly significant.

CONTRIBUTIONS OF THE DIFFERENT TERMS IN THE REGRESSION FORMULA

The α_0', α_1', . . . α_5' are the coefficients in the representations (by orthogonal polynomials) of the regression function of yield on rainfall. They are also the partial regression coefficients of yield on the 0th, 1st, . . . 5th coefficients of the orthogonal polynomials fitted to each year's rainfall, and such partial regression coefficients represent, in order, the regression of yield on total seasonal rainfall, on the average rate at which the rainfall was increasing or decreasing during the season, on the parabolic term in the rainfall sequence, and so on to the higher terms of the polynomials fitted to the seasonal rainfall. Each of these regression coefficients in the formula may be tested to see if it differs significantly from zero (*2, sec. 29*). The coefficient α_1', which represents the regression on the linear component of a polynomial, when tested is highly significant. The t value is 3.55 as compared with 2.797 at the 1-percent level for 24 degrees of freedom. Neither coefficient α_0', representing the regression on the mean rainfall for the season, nor the coefficients α_2', α_3', α_4' approach significance. The coefficient α_5^1 approaches the 5-percent level of significance, it having a t value of 1.84 as compared with the tabular value of 2.064. Had yield been studied only in relation to the total rainfall during the season we might have arrived at the misleading conclusion that the total amount affects yield. The present analysis shows that the real effect is from the distribution.

ANALYSIS OF SPRING WHEAT DATA

The analysis of the spring wheat data involves the steps which are necessary in obtaining the regression of yield on the amount and

distribution of rainfall, and also the partial regression of yield on the amount and distribution of evaporation eliminating associated rainfall effect. Whereas six coefficients representing the seasonal amount and distribution of rainfall were used in the corn analysis, only four coefficients are used in the analysis of the spring wheat yields to obtain a regression function of a similar nature.

The decision to use a third-degree polynomial resulted from having first used a fifth-degree polynomial to represent the seasonal amount and distribution of rainfall and finding that the contributions of the fourth and fifth terms were not significant.

The regression of wheat yield on the amount and distribution of rainfall is found first by the method outlined in the previous section.

TABLE 8.—*Average spring wheat yields from two continuously cropped plots at Dickinson, N. Dak., and seasonal rainfall distribution coefficients*

Year	Yield per acre	ρ_0'	ρ_1'	ρ_2'	ρ_3'	Year	Yield per acre	ρ_0'	ρ_1'	ρ_2'	ρ_3'
	Bushels						*Bushels*				
1908	21.0	10.48	3.88	−587.5	−192	1922	21.6	11.67	39.59	−599.2	−3,336
1909	26.0	11.14	35.80	−790.6	−61	1923	11.3	12.23	88.79	219.1	−1,714
1910	17.8	7.92	14.10	−8.2	116	1924	16.6	8.10	45.92	−173.7	−1,324
1911	3.6	4.99	2.37	−357.5	−505	1925	5.8	7.75	18.23	−436.1	−469
1913	17.0	5.43	16.55	−195.0	−996	1926	.0	6.44	6.62	−385.7	−1,104
1914	9.7	16.79	144.57	338.5	5,945	1927	12.4	11.51	−29.61	−248.2	1,447
1915	27.2	13.42	75.48	−479.9	242	1928	13.8	9.28	49.86	−218.4	−3,900
1916	18.8	11.01	12.93	−282.0	−2,068	1929	9.2	7.51	4.71	−776.2	−787
1917	4.1	4.80	4.02	−205.7	−2,040	1930	6.2	7.48	−13.66	−383.2	−1,651
1918	5.4	7.12	1.56	75.9	1,630	1931	1.1	6.41	45.77	−355.3	−3,742
1919	.6	4.74	−38.12	109.2	1,348	1932	14.7	9.76	4.86	−751.4	−1,480
1920	14.2	9.36	51.50	−271.4	−3,197	1933	1.3	7.27	18.23	−194.6	−971
1921	3.2	7.50	25.70	−270.7	−773	1934	.6	5.51	12.85	−296.1	−3,553

Table 8 includes the annual yield values and the annual rainfall distribution coefficients. It is evident that there is a slight downward trend in yields of these continuously cropped plots. In order to eliminate trend, a linear regression line was fitted in all cases; but because of the missing year, 1912, the ξ' values could not be used as in corn, and individual deviations had to be calculated. In table 9 are

TABLE 9.—*Yield and rainfall distribution coefficients from table 8 with linear trend removed*

Year	Yield	ρ_0'	ρ_1'	ρ_2'	ρ_3'	Year	Yield	ρ_0'	ρ_1'	ρ_2'	ρ_3'
1908	3.15	0.72	−30.27	−362.7	−809	1922	11.05	3.05	15.33	−306.6	−2,372
1909	8.67	1.46	2.36	−560.9	−565	1923	1.27	3.69	65.24	516.5	−637
1910	.99	−1.67	−18.63	226.3	−275	1924	7.09	−.36	23.08	128.5	−135
1911	−12.69	−4.52	−29.46	−118.1	−783	1925	−3.19	−.63	−3.91	−129.1	833
1913	1.76	−3.92	−14.06	54.0	−1,048	1926	−8.47	−1.86	−14.81	−73.8	311
1914	−5.02	7.52	114.66	592.4	6,005	1927	4.46	3.29	−50.34	68.5	2,975
1915	13.00	4.23	46.28	−221.2	415	1928	6.38	1.14	29.84	103.1	−2,259
1916	5.12	1.90	−15.56	−18.5	−1,782	1929	2.30	−.55	−14.60	−449.8	967
1917	−9.06	−4.23	−23.77	62.7	−1,641	1930	−.18	−.50	−32.27	−52.0	216
1918	−7.24	−1.83	−25.52	349.1	2,142	1931	−4.76	−1.49	27.87	−19.2	−1,762
1919	−11.52	−4.13	−64.50	387.2	1,973	1932	9.36	1.94	−12.34	−410.5	613
1920	2.61	.57	25.83	11.5	−2,459	1933	−3.52	−.47	1.74	151.1	1,234
1921	−7.87	−1.21	.74	17.0	78	1934	−3.69	−2.14	−2.93	54.5	−1,235

shown the yields and rainfall coefficients with trend removed, and in future calculations these deviations from linear trend are used instead of the actual values.

The sums of products of deviations from linear trend of yield and rainfall distribution coefficients are:

$S(y\rho_0') = 310.0348$ $S(y\rho_2') = -23{,}121.527$
$S(y\rho_1') = 1{,}655.3187$ $S(y\rho_3') = -81{,}791.340$

The sums and products of deviations of rainfall distribution coefficients appear in table 10. Table 11 displays the rainfall multipliers.

TABLE 10.—*Sums of squares and products of deviations of rainfall coefficients*

Variable	ρ_0'	ρ_1'	ρ_2'	ρ_3'
ρ_0'	209.7418	1,798.6864	634.926	45,413.89
ρ_1'	1,798.6864	34,787.8882	77,137.194	217,110.13
ρ_2'	634.926	77,137.194	2,005,019.60	5,140,831.1
ρ_3'	45,413.89	217,110.13	5,140,831.1	87,699,870

TABLE 11.—*Values of C_{rs}(rainfall) from the sums of squares and products in table 10*

r \ s	C_{r0}	C_{r1}	C_{r2}	C_{r3}
C_{0s}	0.0116104116	$-0.0^{6}64333920$	$0.0^{3}38137625$	$-0.0^{6}66551739$
C_{1s}	$-.0^{6}64333920$	$.0^{6}67079232$	$-.0^{3}33015510$	$.0^{6}36061272$
C_{2s}	$.0^{3}38137625$	$-.0^{3}33015510$	$.0^{5}75716799$	$-.0^{7}55959632$
C_{3s}	$-.0^{6}66551739$	$.0^{6}36061272$	$-.0^{7}55959632$	$.0^{7}17236327$

These multipliers, or C values, are calculated as outlined in a previous section of this report. The regression of yield on rainfall is then obtained by myltiplying the sums of cross products of yield and rainfall distribution coefficients times the appropriate multipliers from table 11, according to equation (8) as follows:

$$310.0348\ (0.0116104116) + 1{,}655.3187\ (-0.0^{6}64333920) + (-23{,}121.527)\ (0.0^{3}38137625) + (-81{,}791.34)\ (-0.0^{5}66551739) = 2.19723569$$

$$310.0348\ (-0.0^{6}64333920) + 1{,}655.3187\ (0.0^{6}67079232) + (-23{,}121.527)\ (-0.0^{5}33015510) + (-81{,}791.34)\ (0.0^{6}36061272) = -0.04157813$$

$$310.0348\ (0.0^{4}3813\ 7625) + 1{,}655.3187(-0.0^{5}33015510) + (-23{,}121.527)\ (0.0^{6}75716799) + (-81{,}791.34)\ (-0.0^{7}55959632) = -0.00657100$$

$$310.0348\ (-0.0^{5}66551739) + 1{,}655.3187\ (0.0^{6}36061272) + (-23{,}121.527)\ (-0.0^{7}55959632) + (-81{,}791.34)\ (0.0^{7}17236327) = -0.00158232$$

As already explained, these four regression coefficients are used to estimate the average effect on the crop in bushels per acre of an extra inch of rainfall occurring within the 120-day period. Figure 2 shows the course of this function.

The regression of wheat yield on rainfall having been found, we turn our attention to the evaporation records. These are to be treated exactly as were the rainfall records, that is, four distribution coefficients, E_0, E_1, E_2, E_3, representing the evaporation for the twenty-four 5-day periods for each season were calculated by fitting a third-degree polynomial to the evaporation sequence of each season. The actual values and deviations from linear trend of the four series of distribution coefficients are shown in tables 12 and 13, respectively.

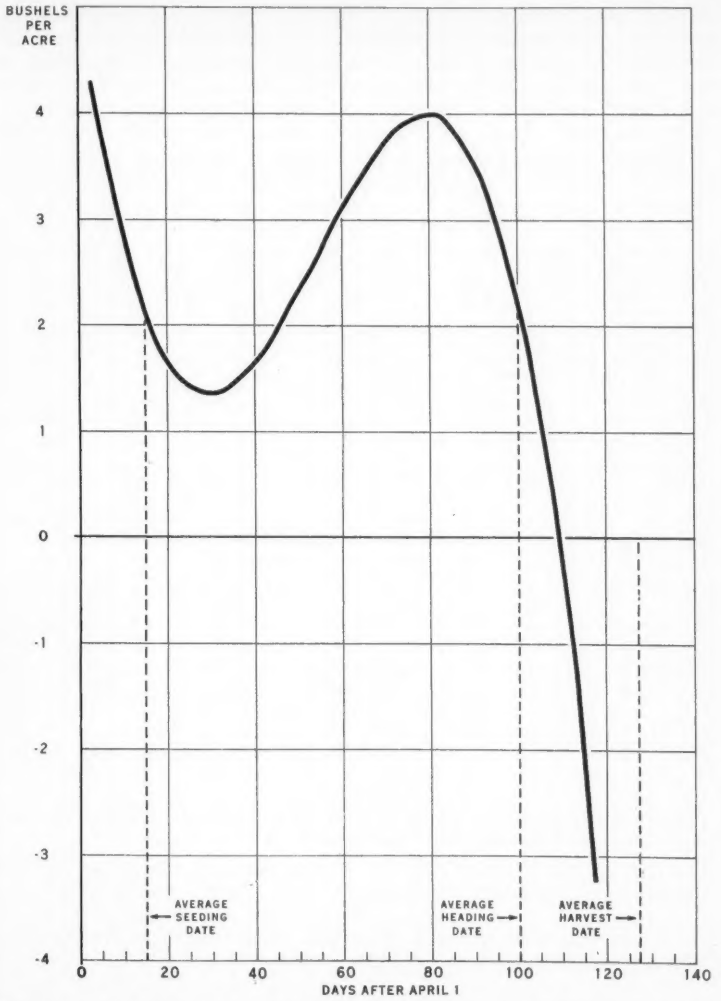

FIGURE 2.—Average effect of an additional inch of rainfall on the yield of spring wheat from continuously cropped plots at Dickinson, N. Dak.

The partial regression of yield on evaporation, eliminating associated rainfall effect, is determined as outlined by Tippett (*7*) and Hopkins (*5*). The associated rainfall effect must be removed from the evaporation values, as must also that portion of the variation in yield that is due to rainfall. The residual evaporation values are then correlated with the yield residuals to give the desired relationship

between yield and evaporation with the associated rainfall effect removed. It is well to recognize here that instead of now using this additional factor, handled in much the same manner as rainfall, we might have used a single factor, had we wished, such as the amount of moisture available in the soil at the beginning of the season.

TABLE 12.—*Evaporation distribution coefficients, Dickinson, N. Dak.*

Year	E_0	E_1	E_2	E_3	Year	E_0	E_1	E_2	E_3
1908	20.591	81.947	71.495	−368.841	1922	18.913	40.457	−325.853	814.859
1909	18.084	39.492	−37.416	518.804	1923	20.647	74.721	−352.811	−419.713
1910	25.855	79.529	215.941	−1,889.197	1924	18.220	67.542	102.226	967.524
1911	27.081	71.259	138.837	−1,103.117	1925	23.974	75.048	−52.898	−769.344
1913	21.831	71.171	−15.831	−775.983	1926	25.347	70.593	−55.773	1,884.341
1914	20.746	80.288	39.958	−1,444.484	1927	18.507	69.313	42.237	592.551
1915	18.508	.792	93.772	−1,397.716	1928	19.585	25.653	−217.655	2,984.581
1916	17.718	42.270	−82.212	419.920	1929	22.828	97.140	140.266	1,781.810
1917	23.716	137.874	−59.312	591.918	1930	25.750	126.700	393.322	−1,346.700
1918	21.415	69.221	−323.603	−70.993	1931	28.690	81.638	−75.806	2,824.866
1919	29.259	130.223	−266.649	−1,339.669	1932	22.496	109.736	367.790	573.462
1920	18.584	52.860	−281.080	1,496.780	1933	26.435	139.039	178.379	−1,733.637
1921	24.089	99.495	148.049	−693.185	1934	31.666	57.182	−198.356	2,653.604

TABLE 13.—*Evaporation distribution coefficients from table 12 with linear trend removed*

Year	E_0	E_1	E_2	E_3	Year	E_0	E_1	E_2	E_3
1908	0.14	20.46	110.1	603	1922	−3.91	−36.86	−311.1	575
1909	−2.54	−23.13	−.5	1,404	1923	−2.35	−3.73	−339.7	−746
1910	5.06	15.78	251.1	−1,090	1924	−4.94	−12.05	113.6	555
1911	6.12	6.38	172.3	−391	1925	.64	−5.67	−43.2	−1,268
1913	.53	4.03	14.2	−237	1926	1.85	−11.26	−47.8	1,299
1914	−.72	12.02	68.3	−992	1927	−5.16	−13.67	48.5	−79
1915	−3.13	−68.61	120.4	−1,032	1928	−4.26	−58.46	−213.1	2,226
1916	−4.09	−28.26	−57.2	700	1929	−1.18	11.90	143.1	937
1917	1.74	66.20	−36.0	785	1930	1.57	40.33	394.5	−2,278
1918	−.73	−3.58	−302.0	36	1931	4.34	−5.87	−76.3	1,807
1919	6.94	56.29	−246.8	−1,319	1932	−2.02	21.10	365.6	−531
1920	−3.90	−22.20	−262.9	1,430	1933	1.75	49.27	174.4	−2,924
1921	1.44	23.31	164.5	−846	1934	6.81	−33.72	−204.0	1,376

The sums of products of deviations from linear trend of yield and evaporation distribution coefficients are:

$S(yE_0) = -\ 486.2268$ $\quad S(yE_2) = 3{,}251.988$

$S(yE_1) = -3{,}376.4161$ $\quad S(yE_3) = 41{,}339.910$

The sums of squares and products of evaporation distribution coefficients are shown in table 14.

TABLE 14.—*Sums of squares and products of evaporation distribution coefficients*

r \ s	E_{s0}	E_{s1}	E_{s2}	E_{s3}
E_{r0}	335.8926	1,478.2213	2,156.183	−27,277.65
E_{r1}	1,478.2213	26,603.5280	61,226.360	−530,147.71
E_{r2}	2,156.183	61,226.360	1,041,705.66	−2,974,677.2
E_{r3}	−27,277.65	−530,147.71	−2,974,677.2	40,898,284.

To obtain the residual sums of squares and products of the 4 evaporation coefficients with the association of the rainfall coefficients removed, we calculate the regression of the evaporation coefficients on the rainfall coefficients. For the regression of the evaporation coefficients on the rainfall coefficients we obtained the 16 regression coefficients in table 16. These are obtained by multiplying the appropriate figures in table 15 of cross products times the appropriate rainfall multipliers from table 11 in the manner indicated in equation (8). For convenience, the regression coefficient of E_s on ρ_r' is designated by β_{rs}. The 16 regression coefficients of table 16 are used in the following equations to obtain the residual sums of squares and products of the 4 evaporation coefficients with the association of the rainfall coefficients removed:

TABLE 15.—*Sums of cross products of rainfall distribution coefficients and evaporation distribution coefficients*

r \ s	ρ_0'	ρ_1'	ρ_2'	ρ_3'
E_0	−177.2879	−1,155.0458	4,856.3770	6,105.86
E_1	−1,027.3392	−11,907.4569	48,279.3420	448,731.49
E_2	−194.602	−33,971.156	−484,275.46	2,402,066.00
E_3	−4,802.38	86,331.47	−2,723,613.40	−30,737,685.00

TABLE 16.—*Regression of evaporation distribution coefficients on rainfall distribution coefficients*

Variable	E_0	E_1	E_2	E_3
ρ_0'	−1.1707241	−5.4124242	−14.859716	−10.605535
ρ_1'	.02274494	−.13539392	.31151172	6.7883564
ρ_2'	.00038751	.01157752	−.39636077	−.81034246
ρ_3'	.00059084	.00757593	.05754736	−.31429949

$$S'(E_s^2) = S(E_s^2) - \beta_{0s}S(\rho_0' E_s) - \beta_{1s}S(\rho_1' E_s) - \beta_{2s}S(\rho_2' E_s) - \beta_{3s}S(\rho_3' E_s)$$
$$S'(E_r E_s) = S(E_r E_s) - \beta_{0s}S(\rho_0' E_r) - \beta_{1s}S(\rho_1' E_r) - \beta_{2s}S(\rho_2' E_r) - \beta_{3s}S(\rho_3' E_r)$$

where $S'(E_s^2)$ is a residual sum of squares and $S'(E_r E_s)$ is a residual sum of products. Such residual sums of squares and products are shown in table 17.

TABLE 17.—*Residual sums of squares and cross products of evaporation distribution coefficients*

r \ s	E_{s0}	E_{s1}	E_{s2}	E_{s3}
E_{0s}	149.0827	259.7954	1,455.047	−15,462.62
E_{1s}	259.7954	15,472.4218	42,982.43	−280,052.25
E_{2s}	1,455.047	42,982.43	719,215.99	−2,383,593.6
E_{3s}	−15,462.62	−280,052.25	−2,383,593.6	28,393,405

The partial regression coefficients of yield residuals on evaporation residuals could be found by using this table directly; however, the

table of multipliers or C values are calculated as before and are shown in table 18.

TABLE 18.—*Values of C_{rs} (evaporation) from the sums of squares and products in table 16*

r \ s	C_{r0}	C_{r1}	C_{r2}	C_{r3}
C_{0s}	0.0071553109	$-0.0^{4}6056141$	$0.0^{6}107969$	$0.0^{5}33084039$
C_{1s}	$-.0^{4}6056141$	$.0^{3}83986663$	$-.0^{5}31320788$	$.0^{5}53246914$
C_{2s}	$.0^{6}107969$	$-.0^{5}31320788$	$.0^{5}20438050$	$.0^{6}14074128$
C_{3s}	$.0^{5}33084039$	$.0^{5}53246914$	$.0^{6}14074128$	$.0^{7}54088115$

The yield residuals are obtained by adjusting the sums of products of yield and evaporation by the product of the regression of evaporation on rainfall and the sums of products of yield on rainfall. These calculations are as follows:

$$-486.2268-310.0348(-1.1707241)-1,655.3187(0.02274494)-(-23,121.527)(0.00038751)-(-81,791.34)(0.00059684)=-103.1356$$

$$-3,376.4161-310.0348(-5.4124242)-1,655.3187(-0.13539392)-(-23,121.527)(0.01157752)-(-81,791.34)(0.00757593)=-586.9207$$

$$3,251.988-310.0348(-14.859716)-1,655.3187(0.31151172)-(-23,121.527)(-0.39636077)-(-81,791.34)(0.05754736)=2,885.7754$$

$$41,339.91-310.0348(-10.605535)-1,655.3187(6.7883564)-(-23,121.527)(-0.81034246)-(-81,791.34)(-0.31429949)=-11,052.24$$

The partial regression coefficients of the yield residuals on the evaporation residuals are then obtained by the same method as outlined previously and are as follows:

$$-103.1356(0.0071553109)+(-586.9207)\ (-0.0^{4}6056141)+2,885.7754\ (0.0^{6}107969)+(-11,052.24)\ (0.0^{5}33084039)=-0.73867623$$

$$-103.1356(-0.0^{4}6056141)+(-586.9207)\ (0.0^{4}83986663)+2,885.7754\ (-0.0^{5}31320788)+(-11,052.24)\ (0.0^{5}53246914)=-0.05797093$$

$$-103.1356(0.0^{6}107969)+(-586.9207)(-0.0^{5}31320788)+2,885.7754\ (0.0^{5}20438050)+(-11,052.24)\ (0.0^{6}14074128)=0.00616959$$

$$-103.1356\ (0.0^{5}33084039)+(-586.9207)\ (0.0^{5}53246914)+2,885.7754\ (0.0^{6}14074128)+(-11,052.24)\ (0.0^{7}54088115)=-0.00084537$$

These partial regression coefficients are used in equation (6) and the course of the regression function for the effect of evaporation throughout the season is obtained as it appears in figure 3.

TABLE 19.—*Analysis of variance of yield of spring wheat*

Source	Degrees of freedom	Sums of squares	Mean square	F
Rainfall regression formula	4	893.7461	223.436	15.30
Residual evaporation regression formula	4	137.3554	34.339	2.35
Deviations from regression formulas	16	233.5717	14.598	.....
Total	24	1,264.6732	.....	.....

Table 19 shows the amount of annual variance in yields accounted for by the rainfall and by the evaporation regression formulas. The mean square due to the rainfall regression formula when compared

with the mean square due to deviations from the regression formulas is highly significant.

In a similar comparison for the residual evaporation regression formula, an F ratio of 2.35 is obtained. Although this value is less

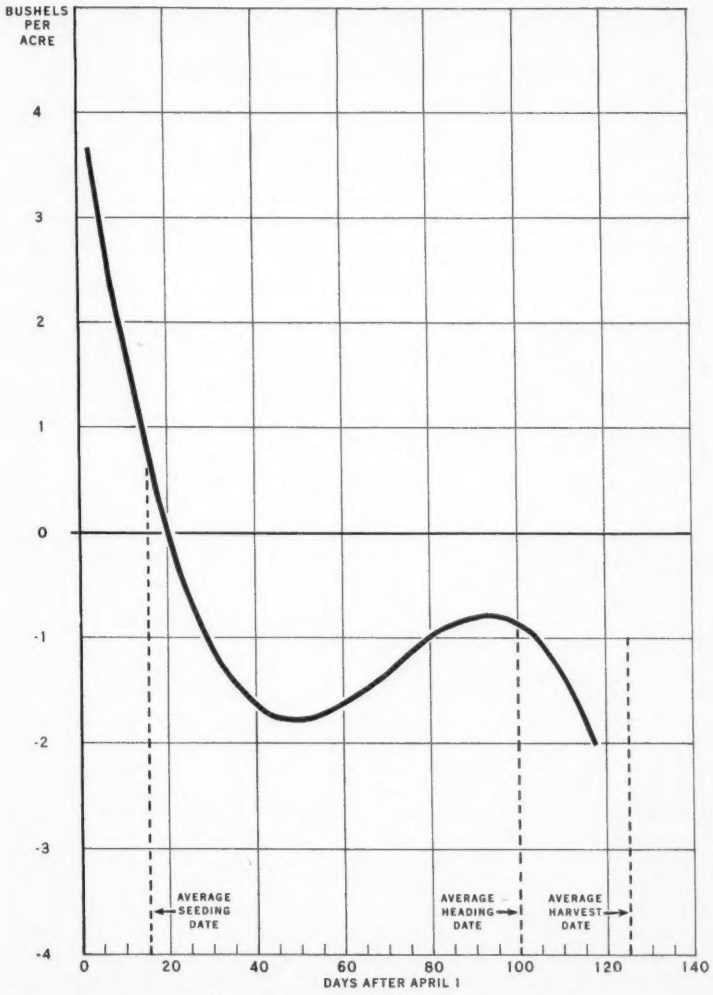

FIGURE 3.—Average effect of an additional inch of evaporation (influence of associated rainfall being eliminated) on yield of spring wheat from continuously cropped plots at Dickinson, N. Dak.

than the F ratio of 3.01 at the usual 5-percent level of significance, it indicates that the effect of additional evaporation as shown in figure 3 may be real. Further support is contained in an unpublished study

of data from additional dry-land experiment stations in which the residual evaporation regression formula was found to be significant. It is therefore not probable that the effects of evaporation occur entirely by chance.

The partial regression coefficients of the various terms in the two regression formulas are shown in table 20 along with the probability

TABLE 20.—*Values and tests of significance of regression coefficients*

Degree	Rainfall regression formula		Evaporation regression formula	
	Regression coefficients	Probability	Regression coefficients	Probability
0	2.19723569	$P<0.001$	−0.73867623	$0.03<P<0.05$
1	−.04157813	$0.2<P<.3$	−.05797093	$.1<P<.2$
2	−.00657100	$.05<P<.1$	.00616357	$.2<P<.3$
3	−.00158232	$.01<P<.02$	−.00084537	$.3<P<.4$

of these occurring by chance. It should be noted that the partial regression of yield on the total evaporation for the season represented by the zero term of the formula is significant.

The coefficients of multiple correlation between yield and rainfall and between yield and rainfall and evaporation are represented by the values of R equals 0.82 and 0.90 respectively.

DISCUSSION

The results for plot 5 of continuous corn grown at Wooster show clearly that although the total rainfall for the season is not significantly correlated with yield, the distribution of the seasonal rainfall is correlated with yield. In terms of the polynomials fitted to seasonal rainfall, it is the linear component that is significantly correlated with yield. This is evident from the general upward slope of the curve which indicates that for corn the effect on yield of an additional inch of rain increases with time over the period studied. In general, those familiar with the crop would expect this, although, so far as the writers are aware, no quantitative expression has heretofore been produced. As the crop progresses and the plants become larger with increased surface for transpiration, one would expect a favorable response to increased quantities of rainfall. Conversely, as may be interpreted from the function, in the period from planting to near maturity, the later in the season a deficit in rainfall occurs, the greater the detrimental effect. Frequently, adverse conditions early in the life of the corn plant are overcome as the season progresses. The fact, that, for the first one-third of the period covered, the curve extends below the zero line indicates that the mean rainfall at Wooster for this part of the season is greater than the optimum for corn.

Importance cannot be attached to fluctuations of the function a about its linear trend upward because, as pointed out in the analysis of the contributions of the several terms to total variation accounted for by the regression formula, none of the other terms has a significant partial regression coefficient. Bearing in mind the unimportance of the other terms of the regression formula, there exist, however,

agronomic facts of common knowledge that suggests the meaning of some of these fluctuations. During the early part of the life of the corn plant its requirement for moisture is limited to an amount sufficient for germination and the small degree of growth that takes place. This amount of moisture is seldom lacking at Wooster at this time. Furthermore, weeds must be kept from the corn, and to make this possible, the soil must be dry enough for cultivation. Any increased amount of rain hinders this. Wet weather is also conducive to weed growth. The amount of nitrogen available to the plant is thought to be enhanced by a warm aerated soil, a condition that cannot exist with large amounts of rainfall. Additional rainfall at this time may hinder root growth—not only the total amount of growth but also the character of it. If the weather is somewhat dry at this time, the roots will penetrate deeper into the soil and thus produce a condition that will better enable the plant to withstand dry weather later. These facts seem to lend support to the downward fluctuations of the curve immediately following the time of planting.

Once the plant is well started, with many of its structural parts laid down and freed from the competition of weeds, it rapidly elongates and responds to increasing amounts of rainfall, as is evidenced by the rapid rise in the curve. The peak of the curve is reached in about 80 days from the beginning of the season and occurs slightly earlier in relation to the average date of silking than might be expected. A great deal has been written concerning a critical period for crops at the time of pollination. Although the peak of the curve is not centered on this period, increased rainfall immediately preceding silking has a very beneficial effect and can be considered as placing the plant in a favorable condition for entering the reproductive stage. The position of the peak is determined somewhat by the character of the curve fitted, and the exact point where the effect of an additional inch of rainfall fails to exceed the effect in the period immediately preceding remains indeterminate. It should be emphasized that the tails of the curve, and even the points on the curve a slight distance from the tails, are regulated by the nature of the curve fitted and relatively little importance should be attached to the extremities of the curve.

The results of the study of the spring wheat crop grown in a more or less arid climate are in sharp contrast to those with corn grown in a humid climate. There is a high correlation between the total seasonal rainfall and yields of spring wheat, whereas in the case of corn the total seasonal rainfall has little association with yields. The average rate of increase or decrease in rainfall from the beginning to the end of the season is not significantly correlated with spring wheat yields, yet in the case of corn the results show that over the period studied the yields are higher if the rainfall increases at a constant rate during the season. Another contrast is that the parabolic and cubic terms in the rainfall sequence are important in the case of wheat but relatively unimportant in the case of corn. It is recognized that had the season been extended for corn the effect of additional rain would have diminished toward the time of harvest. Furthermore, the continuous corn series used may reflect a greater benefit from additional rainfall toward the end of the season than would corn on rotated

land. Nevertheless, it appears that for wheat the effect of additional rain rapidly diminishes from the time of heading. This appears to be an inherent biological characteristic of wheat.

The rainfall curve displayed in figure 2 would indicate that additional rainfall at any time during the growing season causes an increase in yield. The greatest beneficial effect is from rain that comes during the rapid growing period of the plant, reaching a maximum about 3 weeks before average heading date. At this time of the season each additional inch increases the yield about 4 bushels per acre.

The fact that these results indicate a rather definite period when additional rainfall has a much greater effect on yield than during the rest of the season, whereas Hopkins' results showed less evidence of this, needs consideration. Hopkins (*5*) recognized, however, that his results are not necessarily incompatible with the existence of a relatively critical period of growth by pointing out that any such period may be overshadowed by conditions of the soil and the amount of moisture present at the beginning of the season.

The problem of what degree polynomial to use in representing sequences of weather when studying spring wheat deserves comment at this point. As previously mentioned, the fourth and fifth terms in a polynomial fitted to seasonal rainfall at Dickinson were not significantly correlated with spring wheat yields. It would appear from the work of Hopkins (*5*) that in case of his Marquis series and rainfall, anything inclusive of the parabolic term would be adequate. The same is true for his stubble series, and here it is even highly questionable whether anything more than the total rainfall is correlated with yield. It appears, therefore, from this and the preceding paragraphs, that the quantity a, representing the average benefit to the spring wheat crop in bushels per acre per inch of rain falling in the time element considered, is a slowly changing function that can be easily represented by polynomials of the third degree or possibly lower.

It is concluded that, while yield appears significantly related to the total seasonal evaporation, the effect of the seasonal distribution of evaporation is not conclusively shown. However, the results are not unreasonable. Figure 3, showing the effect of the amount and distribution of evaporation on spring wheat yields, when associated rainfall effects are eliminated, indicates that above-average evaporation is detrimental to the crop over most of the growing season. Early in the season high evaporation, partly a result of above-average temperatures, would be favorable for obtaining a good stand of wheat. Later in the season, after the heading, increased evaporation may hasten ripening and cause a lowering of yield.

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DEVELOPMENT OF THE STAMINATE AND PISTILLATE INFLORESCENCES OF SWEET CORN[1]

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INTRODUCTION

Studies of the morphology of the corn plant have led, according to Weatherwax (*17*),[2] to clearer and simpler explanations of the results of certain experiments with corn. It should be expected that further studies of the developmental morphology might prove to be equally helpful. Variations from the normal sequence of development which lead to abnormalities of the tassel and ear and variations in such characters as length of ear, number of rows of kernels on the ear, irregularities in the straightness of the rows of kernels, and correlation in the development of the ears of multiple-eared types, to mention a few examples, can be better understood by studying the developmental morphology. By means of frequent observations during the development of the growing points it is possible to see whether the variations observed in the mature plant are the result of variation in the pattern of differentiation and development or are the result of growth responses to changes in the environment.

While excellent descriptions of the morphological development of certain of the parts of the inflorescences have been written, some of which will be cited later, few workers have attempted to describe and illustrate all of the steps in the ontogeny of the staminate (tassel) and pistillate (ear) inflorescences. In this paper the major steps in the development of the tassel and ear from the undifferentiated growing points of the shoots to the fully differentiated flowers and flower parts are described and illustrated with photomicrographs.

LITERATURE REVIEW

Only a few of the publications dealing with the morphology of the corn plant will be reviewed. Most of the authors cited have made extensive reviews of the literature. In this connection special attention is called to the publications of Weatherwax (*15*), Miller (*8*), Randolph (*11*), and Arber (*1*).

The general morphology of the tassel and ear of the corn plant has been described by Collins (*5*), Weatherwax (*15, 16, 18*), and Arber (*1*). A clear understanding of the development of the pistillate spikelet is given by Miller (*8*), and Randolph (*11*) has described and illustrated the development of the pistillate spikelet and the caryopsis. Schuster (*12*) has described and illustrated some of the early stages of spikelet development.

Noguchi (*9*) is the only one of those cited who has described some of the beginning stages in the development of the ear and tassel, and he has illustrated a few of these stages with drawings.

[1] Received for publication May 22, 1939.

[2] Italic numbers in parentheses refer to Literature Cited, p. 36.

Descriptions of many-flowered spikelets, seeds in the tassel, and other variations from normal development in the inflorescences have been published by Kempton (*6*), Stratton (*13*), and Weatherwax (*18*).

MATERIALS AND METHODS

First-generation hybrids, Golden Cross Bantam (Purdue Bantam 39 × Purdue 51) and a Country Gentleman hybrid (Illinois 8 × Illinois 6) were used in these studies. Sweet corn (*Zea mays* var. *rugosa* Bonafous) was used because it could be easily grown in the greenhouse and, with an early-maturing type like Golden Cross Bantam, it was possible to get the various stages of development in a relatively short time. Another reason for using hybrids was that there would probably be less variation in development among individual plants which would make it easier to follow the developmental sequences.

In a study of the developmental morphology of the caryopsis, Randolph (*11*) found no significant differences among dent, flint, and sweet corn. Likewise, no essential differences were noted in the morphological characteristics of the inflorescences of dent and sweet corn. Therefore, it seems reasonable to expect that whatever is found out regarding the ontogeny of the inflorescences of sweet corn would also apply to dent corn.

The corn plants were grown in the greenhouse, without artificial lights, in glazed 2-gallon gars, filled with a mixture of sand, soil, and well-rotted stable manure.

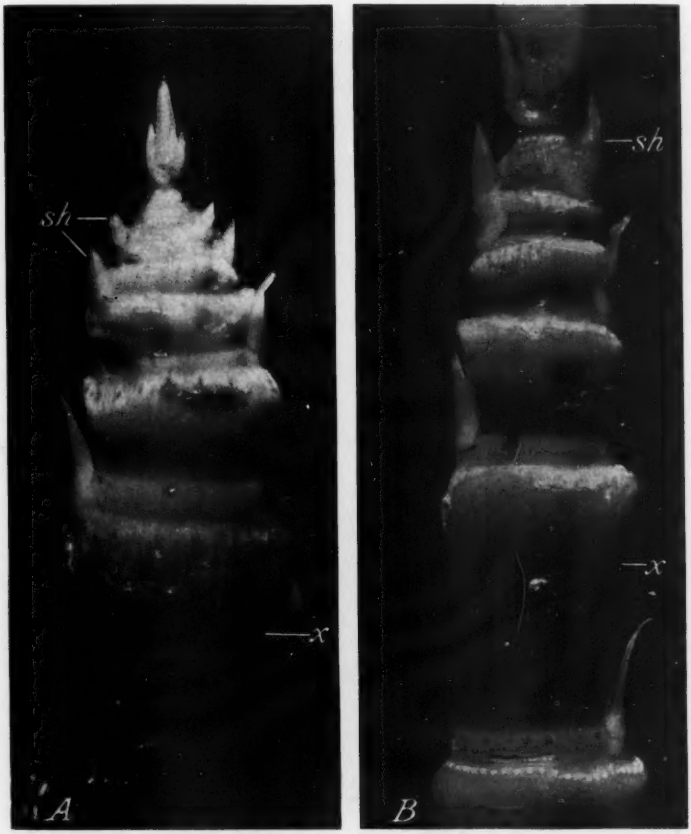
Growing points at successively later stages of development were removed from the plants and photomicrographs were taken. The photographic set-up was essentially the same as one already described (*2*). Photomicrographs were taken with an upright camera and special microlenses having focal distances of 16, 24, 32, and 48 mm. Light for photographing was obtained from a microscope lamp fitted with a 200-watt bulb. A Florence flask filled with distilled water was used as a condenser.

In order to bring out the morphological details in some of the specimens, a stain composed of a mixture of 90-percent alcohol, a small amount of glycerin, and basic fuchsin was applied. The alcohol quickly evaporated, leaving the stain and glycerin in the folds of the various structures which made them more easily seen.

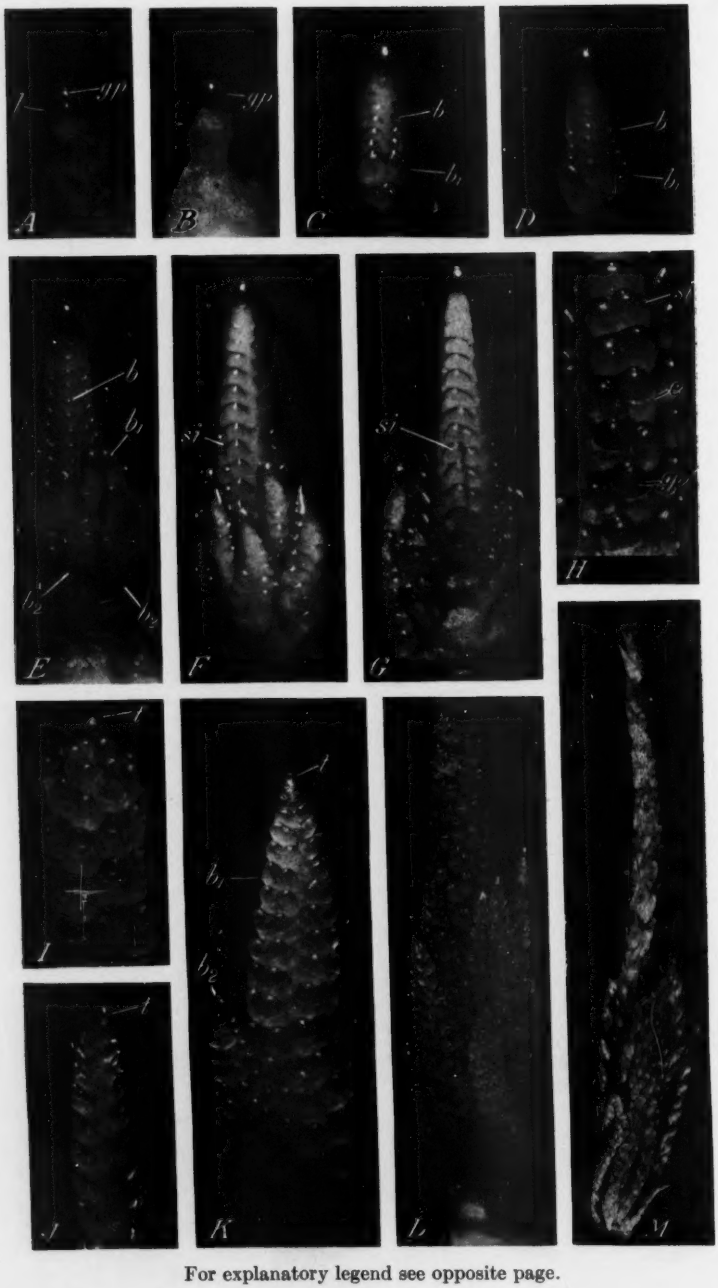
DEVELOPMENT OF THE INFLORESCENCES

Usually when the plants had 8 to 10 leaves, the ear shoot and tassel had begun to form (fig. 1). However, the number of leaves that a plant has is not a reliable guide to the stage of development of the inflorescence. Plants with the same number of leaves may differ to a considerable extent in the degree of development of the growing points owing to differences in growing conditions, differences in variety, and other factors.

Tassel and shoot development in a plant that was at the same stage of development as those shown in figure 1 is illustrated in plate 1, *A*. The tassel has formed, the axillary shoots from which the ears will develop (pl. 1, *A*, *sh*, and *B*, *sh*) can be seen on the upper part of the stem, and the basal internodes of the stem have begun to elongate (pl. 1, *A*, *x*, and *B*, *x*).



Stems of Golden Cross Bantam sweet corn with leaves removed to show the tassel, shoots, nodes, and internodes. *A* is an earlier stage than *B*; *x*, Intermodes; *sh*, axillary shoot. × 5.



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TASSEL AND STAMINATE SPIKELET DEVELOPMENT

The shoot of the corn plant, like that of other cereals (*2, 3, 4*) and grasses (*20*), passes through two stages in its development from germination to the dehiscence of the anthers. During the first stage, leaf fundaments, leaves, and axillary shoots are produced and the internodes of the stem remain short. During the second stage the internodes of the stem elongate, the tassel and its parts differentiate and develop, and the axillary shoot or shoots (ear or sucker) pass through their various stages of development.

Two growing points (pl. 2, *A* and *B*) represent the appearance of the shoot in the first stage of development. The growing point (pl. 2, *A*, *gp*) is partly enclosed by two leaf initials. At this stage of development the growing point is much smaller in relation to the diameter of the stem than the growing point of either wheat, oats, or barley at a similar stage of development.

Two processes, which occur simultaneously, indicate the beginning of the second stage of development. (1), The internodes of the stem begin to elongate (pl. 1, *A*, *x*, and *B*, *x*), and (2) the growing point elongates in preparation for the differentiation of the tassel and its parts. During the second stage the growth activities are internode elongation and the differentiation of the tassel and its parts. Tassel development is completed when the anthers dehisce.

Branch primordia are the first of the tassel parts to differentiate (pl. 2, *C*, *b*, and *D*, *b*). They arise in acropetal succession as lateral projections from all sides of the elongated central axis. Some of the branch initials at the base of the central axis elongate and become the lateral axes of the tassel (pl. 2, *E*, b_1). The other initials arising from a point higher on the central axis are the initials from which the spikelet initials originate (pl. 2, *C*, *b*; *D*, *b*; *E*, *b*; *F*, *si* and *G*, *si*).

It should be noted that, in the early stages of development of the tassel, so far as external appearances indicate, there are no differences between those initials that become the lateral branches of the first order and those from which the spikelet initials differentiate. Therefore all of the first initials to appear are branch initials.

Explanatory Legend for Plate 2

A.—Growing point of a corn plant having four leaves visible. × 22.

B.—Beginning of the elongation of the growing point just before tassel differentiation. × 22.

C and *D*.—Differentiation of the branches of the tassel × 22.

E.—Elongation of the basal branches of the tassel. × 22.

F.—Beginning of the differentiation of the spikelet initials on the central axis of the tassel. × 22.

G.—A stage similar to *F* with some of the basal branches removed to show spikelet differentiation on the central axis. × 22.

H.—Differentiation of spikelets and empty glumes on a portion of the central axis of the tassel. × 35.

I.—Portion of the tip of the central axis of the tassel. × 25.

J.—Adaxial side of a branch of the tassel. × 22.

K.—Abaxial side of tassel branches of the first and second orders. × 25.

L.—A more advanced stage of tassel development. × 22.

M.—Fully differentiated but not full-sized tassel. × 8.

b. Branch initial from which spikelets differentiate; b_1, basal branch of the first order; b_2, branch of the second order; *e*, empty glumes; *gp*, growing point; *gp'*, growing point of the spikelet; *l*, leaf initial; *si*, spikelet initial; *t*, undifferentiated tip of an axis.

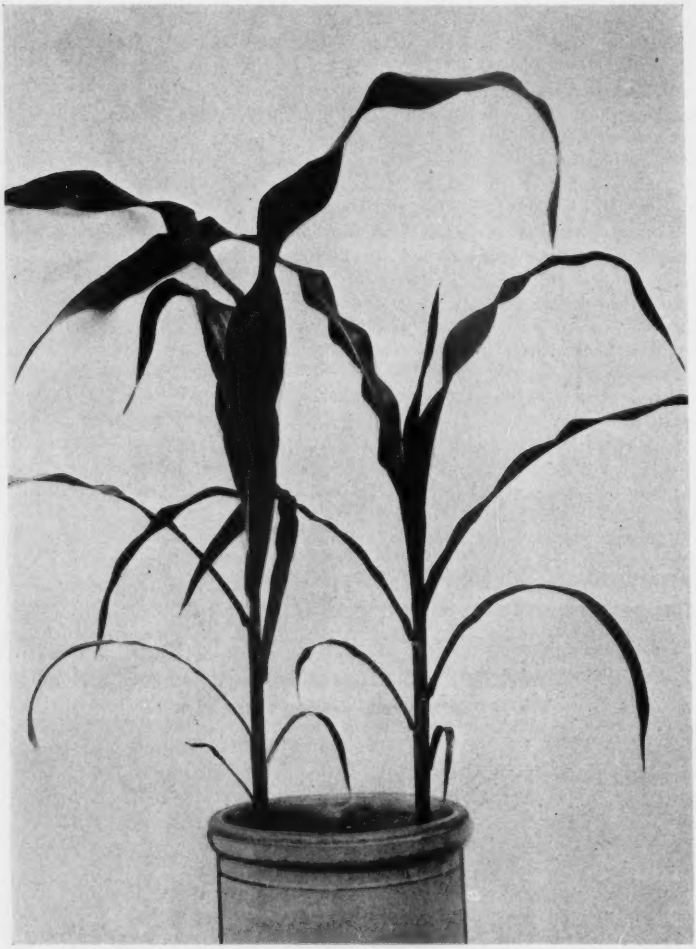
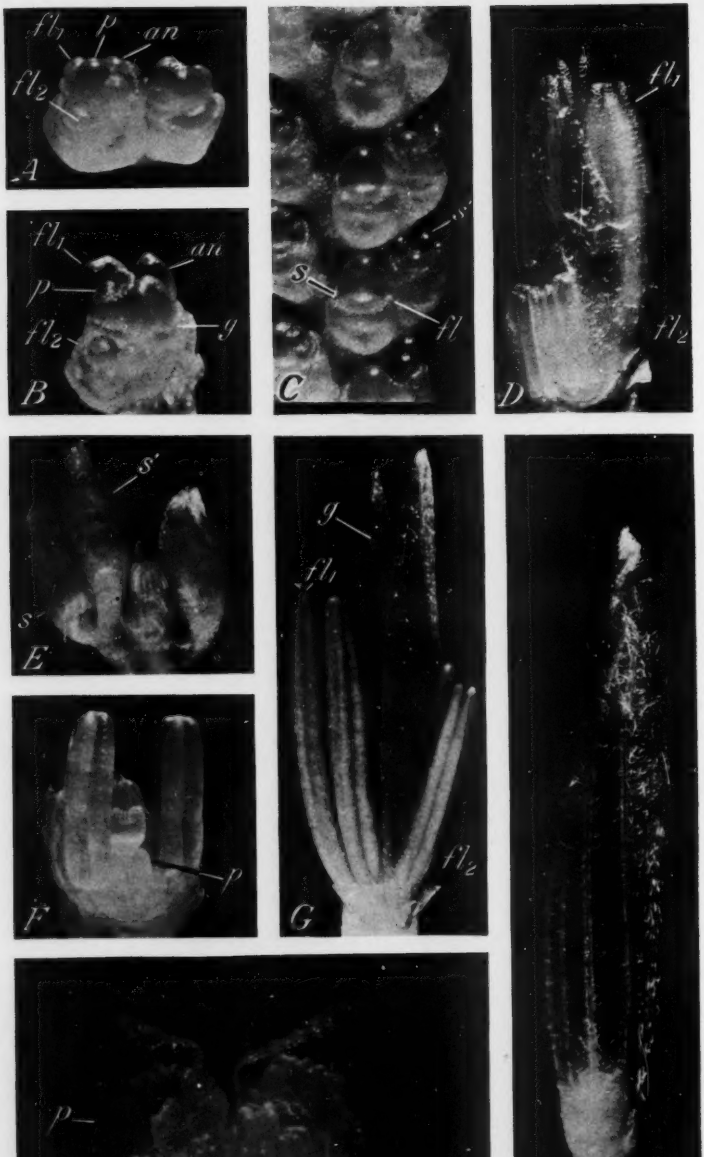


FIGURE 1.—Plants of Golden Cross Bantam sweet corn at the same stage of development as the dissected plants shown in plate 1, A and B.

As has been described for oats (*4*), branches of the second order may rise by budding from the base, and at the lateral margins, of the branches of the first order (pl. 2, *E*, b_2, and *K*, b_2). As has already been stated for the central axis, those initials of the lateral axes above the most basal ones are the primordia from which the spikelet initials differentiate.

In studies made on barley, wheat, and oats (*2*, *3*, *4*) there was always an indication of leaf fundaments on the central axis in the axils of which the lateral shoots of the inflorescence were formed. There is, however, no indication of leaf fundaments subtending the initials



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of the lateral shoots of the tassel, but, as will be pointed out later, there are structures apparently homologous with leaf initials, subending the lateral shoots of the ear (pl. 4, *C*, *l*).

All the branches of the tassel are indeterminate. Neither the central axis (pl. 2, *I*, *t*) nor the lateral axes (pl. 2, *J*, *t*, and *K*, *t*) of the tassel terminate in apical spikelets. Primordia from which the spikelet initials differentiate are produced acropetally as long as the axes increase in length.

In the beginning of spikelet development the branch initial divides into two unequal parts, the spikelet initials (pl. 2, *F*, *si*; *G*, *si*; and *H*, *si*). The spikelet that develops from the larger spikelet initial is pediceled (pl. 3, *E*, *s'*) and the spikelet from the smaller spikelet initial is sessile (pl. 3, *E*, *s*). The larger initial is always in advance of the smaller in its development. This is shown by the beginning of development of the empty glume on the larger initial in plate 2, *H*, *e*, and the lack of such development in the smaller initial and by the beginning of anther differentiation in the larger spikelet in plate 3, *C*, *s'*, and the lack of anthers in the smaller spikelet plate 3, *C*, *s*.

Several of the early stages of development of the spikelets can be seen in plate 2, *H*, which shows a group of spikelets from the central axis of the tassel. The stages of development beginning at the top of the photograph range from an undifferentiated lateral shoot initial, through the various stages of division into spikelet initials, to the beginning of development of the empty glumes. The empty glumes are the first of the spikelet parts to form and are first seen as transverse ridges across the spikelet initial (pl. 2, *H*, *e*). They grow in length and finally enclose the flowers (pl. 3, *I*).

Spikelet initials develop from all sides of the central axis of the tassel (pl. 2, *G*) but only on the abaxial side of the lateral branches. The abaxial side of branches of the first and second order are shown in plate 2, *K*, b_1, b_2, and the adaxial side of a branch is shown in plate 2, *J*. Two rows of lateral shoot primordia develop and they divide into two pairs of spikelet initials.

At any stage of development the central axis of the tassel is in advance of the branches (pl. 2, *F*, *L*, and *M*). This is what should be expected since the central axis is formed first and the branches

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EXPLANATORY LEGEND FOR PLATE 3

A.—Two spikelets of the tassel at the beginning of the development of the flower parts of the upper flowers and the more advanced stage of development of the spikelet at the left. × 56.

B.—Two flowers of a spikelet of the tassel showing the more advanced stage of development of the upper flower. × 56.

C.—Part of a branch of the tassel showing a more advanced stage of development of the pedicled spikelet. × 40.

D.—Staminate spikelet with the empty glumes removed to show the difference in the size of the anthers of the upper and lower flower. × 20.

E.—Two pairs of spikelets, one member of each pair is pediceled and the other sessile, the empty glumes have been removed from one spikelet. × 25.

F.—Staminate flower with one anther removed to show the partly developed pistil. × 20.

G.—Later stage of spikelet development in which the anthers of the lower flower are approaching the size of those of the upper flower. × 10.

H.—Pair of spikelets both sessile. × 28.

I.—Fully differentiated spikelet. × 10.

an, Anther; *fl*, flower initial; fl_1, upper flower; fl_2, lower flower; *g*, palea; *p*, pistil; *s*, sessile spikelet; *s'*, pediceled spikelet.

differentiate from it. The branches increase considerably in size before the initials from which the spikelets differentiate are produced.

In each staminate spikelet two flowers develop from the meristem located above the empty glume initials (pl. 2, *H*, *gp'*). The meristem divides into two unequal parts. The larger part gives rise to the upper flower (pl. 3, *A*, fl_1, and *B*, fl_1) and the smaller part develops into the lower flower (pl. 3, *A*, fl_2, and *B*, fl_2. These flowers differ in their rates of development. The anthers of the upper flower (pl. 3, *A*, fl_1; *B*, fl_1; *D*, fl_1; and *G*, fl_1) differentiate first and in their development are always ahead of the corresponding parts of the lower flower (pl. 3, *A*, fl_2; *B*, fl_2; *D*, fl_2; and *G*, fl_2). As the flowers approach maturity the anthers of the lower flower attain nearly the same size as the anthers of the upper flower (pl. 3, *G*, fl_1, and fl_2).

Anther initials are the first of the flower parts to differentiate (pl. 3, *A*, *an*, and *B*, *an*). Since the tassel flowers are staminate, anther differentiation and development are the principal growth activities within the flower.

Pistils may develop from the meristem located above the anther initials (pl. 3, *A*, *p*) but they usually remain rudimentary (pl. 3, *B*, *p*). Under certain conditions of growth the pistil may show considerable development (pl. 3, *F*, *p*) and may become fully developed and functional (pl. 7, *E*, *p*).

Flowering glumes develop for each flower, but they are so thin (pl. 3, *G*, *g*) that they are difficult to distinguish at the beginning of their development. The lemma and palea begin their development as thin ridges at a point on the meristem just below the anther initials (pl. 3, *B*, *g*) at about the same time that the anther initials begin to differentiate.

Deviations from the normal development under field conditions are often seen in plants grown in the greenhouse. Normally one spikelet is sessile and the other pediceled (pl. 3, *E*), but both spikelets may be sessile (pl. 3, *H*). Another type of deviation which will be described later is the development of functional pistils in the tassel.

EXPLANATORY LEGEND FOR PLATE 4

A.—Axillary shoot in which the ear develops, enclosed in the prophyllum. × 13.

B.—Side view of an axillary shoot. × 17.

C.—Beginning of the differentiation of the ear. × 25.

D.—Ear development showing a more advanced stage of branch differentiation. × 25.

E.—Beginning of spikelet differentiation by an unequal division of the branch initials. × 22.

F.—Development of the empty glumes. × 22.

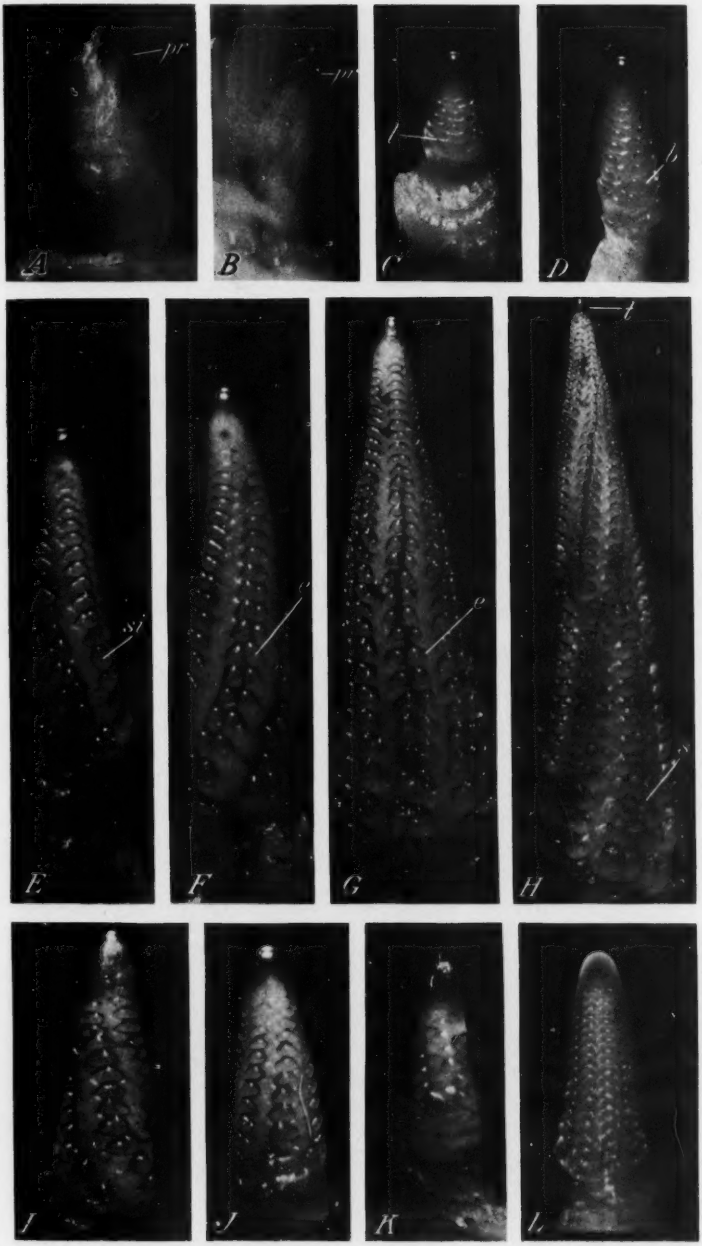
G.—Paired rows of the ear and a more advanced stage of the development of the empty glumes. × 17.

H.—The differentiation and development of the silks can be seen at the base of the ear. × 17.

I, *J*, and *K*.—Topmost, second, and third ears, respectively. All × 22.

L.—Young ear of Country Gentleman sweet corn. × 17.

b, Branch inital from which spikelet initials differentiates; *e*, empty glumes; *l*, leaf fundament; *pr*, prophyllum; *si*, spikelet initial; *s*, silk initial; *t*, undifferentiated tip of the ear.



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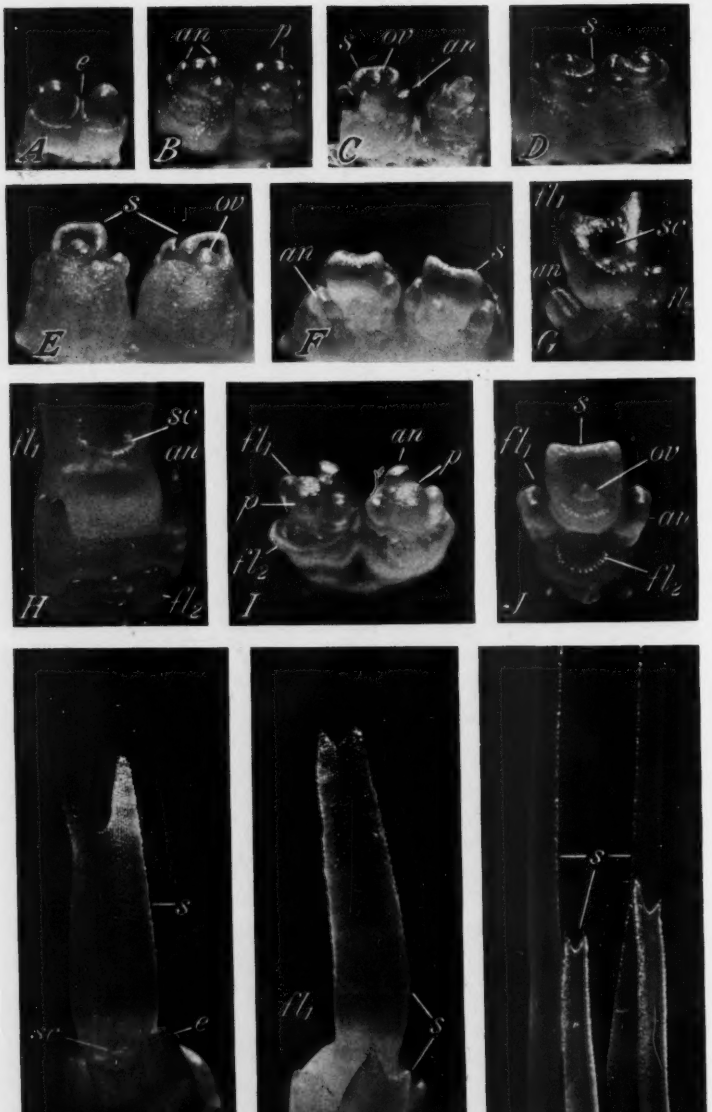

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EAR AND PISTILLATE SPIKELET DEVELOPMENT

In the early stage of stem development a shoot is produced in the axil of each leaf (pl. 1, *A*, *sh*, and *B*, *sh*), but at a later stage of development axillary shoots are no longer produced. The cessation of axillary shoot development seems to be associated with the elongation of the internodes of the stem and the development of the tassel. This is in agreement with observations made by Percival (10) on the cessation of axillary shoot (tiller) development in wheat and the same thing has been observed regarding axillary shoot development in barley.[3]

Ears develop from the upper one or more axillary shoots of the stem. Those shoots formed at the base of the stem may remain nonfunctional or develop into suckers. If an examination is made at the time the topmost shoots are producing ear initials, it will be found that the growing points of the basal shoots are producing only leaf fundaments; but they are more and more advanced in development from the base to the top of the stem.

Axillary shoots develop in acropetal succession and during the early stage of stem development the axillary shoots became larger in succession from the apex to the base of the stem (pl. 1, *A*). Later when the ears begin to develop, the size sequence changes, so that the topmost shoot is the largest and the shoots become smaller from the top to the base of the plant (pl. 1, *B*). The topmost shoot or the topmost two or three shoots, depending upon whether they are single- or multiple-eared types, in turn take precedence in their development or they may inhibit the development of the shoot immediately below. This difference in development is shown by the size of the ear initials in plate 4, *I*, *J*, and *K*, which are the ear initials from the topmost, second, and third shoots, respectively.

[3] BONNETT, O. T. TILLERING IN BARLEY AS INFLUENCED BY CERTAIN PLANT CHARACTERISTICS. 1933. (Abstract of doctor's thesis, Univ. Ill.)

EXPLANATORY LEGEND FOR PLATE 5

A.—Pair of pistillate spikelets at an early stage of development. × 40.

B.—Beginning of the differentiation of anthers in the upper flower of a pair of spikelets. × 40.

C.—Differentiation of a silk, the first stage of pistil development. × 40.

D.—Silk development. × 40.

E.—Silks partly enclosing the ovules. × 40.

F.—Silk development from the adaxial side. × 40.

G.—Spikelet showing the comparative development of the upper and lower flowers. × 40.

H.—Functional upper flower and a sterile lower flower of the spikelet are illustrated. × 40.

I.—Pair of spikelets of Country Gentleman at an early stage of development. × 40.

J.—Spikelet of County Gentleman comparing the development of the functional upper and lower flowers. × 40.

K.—Silk development of the functional flower of a spikelet having only one functional flower. × 28.

L.—Comparison of the silk development of the lower and upper flowers of a spikelet having two functional flowers. × 28.

M.—A more advanced stage in the development of spikelets having two functional flowers. × 10.

an, Anther initial; *e*, empty glumes; fl_1, upper flower; fl_2, lower flower; *ov*, ovule; *p*, pistil initial; *s*, silk initial; *sc*, stylar canal.

The axillary shoot is enclosed in a strongly keeled prophyllum (pl. 4, *A*, *pr*, and *B*, *pr*) which may be entire or divided. Leaf initials that develop into the husks are covered by the prophyllum.

Ear differentiation is indicated by an elongation of the growing point of the axillary shoot and the differentiation of lateral projections from the central axis of the ear initial (pl. 4, *C*, and *D*). The lateral projections are the initials from which the spikelet initials differentiate and correspond to the initials that first appear on the central axis and branches of the tassel. Subtending each initial, as has already been mentioned, are ridges (pl. 4, *C*, *l*) which are similar to the subtending leaf initials that appear in the differentiation of the inflorescences of barley, oats, and wheat. These ridges increase in size and form the cuplike depressions in which the spikelets occur (pl. 6, *B*, *x*).

Spikelet initials are produced in pairs by the division of the preceding initial into two unequal parts (pl. 4, *E*, *si*). While the parts of the larger of the pair of spikelet initials begin to differentiate before those of the smaller spikelet initial, the difference in their development is not so great as was pointed out for the spikelet initials of the tassel.

The empty glumes are the first of the spikelet parts to form and can be seen as transverse ridges across the spikelet initial (pls. 4, *F*, *e*, and 5, *A*, *e*). More advanced stages of development of the glumes are shown in plate 4, *G*, *e*. As the empty glumes increase in length, they enclose the ovary, but the silk extends beyond them (pl. 5, *K*, e).

Straightness of row and the number of rows of kernels per ear are characteristics of the ear that are determined when the spikelets differentiate. Variations in the straightness of row can be seen in plate 4, *E*, *F*, *G*, and *H*. The rotation to the left or right (pl. 4, *E* and *F*) and the regularity or irregularity in the placement of the spikelets (pl. 4, *G* and *H*) can be seen. Row number is determined by the number of rows of branch initials around the ear initial (pl. 4, *D*, *b*) from which a pair of spikelets differentiates. Each spikelet has a fertile flower from which the kernels are produced.

The ear as well as the tassel is indeterminate in its growth and continues to elongate at the tip (pl. 4, *H*, *t*), but many of the flowers at the tip of the ear remain rudimentary (pl. 6, *C*, *t*). Since the spikelets arise in acropetal succession they are successively younger from the base to the tip of the ear (pl. 4, *G* and *H*).

Two flower initials are produced in each spikelet, but in most corn varieties only one flower is functional. In a few types like Country Gentleman sweet corn both flowers are functional.

The two flowers of the ear develop from an unequal division of the meristem of the spikelet just as was pointed out for the flowers of the tassel. The flower differentiating from the larger mass of meristem

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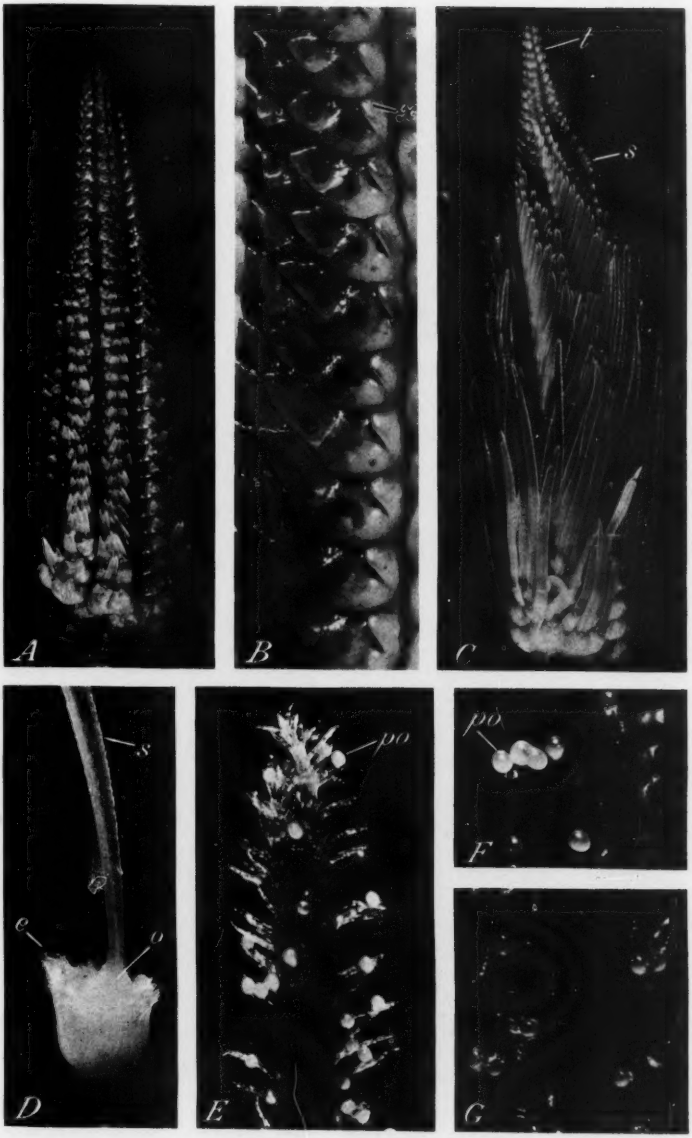
EXPLANATORY LEGEND FOR PLATE 6

A.—Ear showing different stages of silk development. × 6.5.
B.—Section of an ear. × 19.
C.—Variation in silk development. × 6.5.
D.—Pistil from the tassel. × 14.
E.—Tip of a mature silk with pollen grains germinating on it. × 26.
F and *G*.—Pollen grains germinating on the silk. × 38.
e, Empty glumes; *o*, ovary; *po*, pollen grain; *s*, silk; *t*, tip of ear; *x*, enlargement of ridge subtending the spikelets of the ear.



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(the upper flower) takes precedence in its development over the flower from the smaller mass of meristem (the lower flower). The larger flower is the functional flower in those types of corn that have only one functional pistillate flower per spikelet. In these types of corn having two functional pistillate flowers per spikelet, the larger flower is more advanced at every stage in its development than the smaller flower.

Anther initials are the first of the reproductive parts of the flower to differentiate (pl. 5, *B*, *an*). In the pistillate flower the anthers begin differentiation but usually remain small and nonfunctional. Under certain growth conditions and in the genetic type, anther ear, the anthers of the pistillate flower may attain full development. Anthers well enough developed to show the locules are shown in plate 5, *G*, *an*, and *J*, *an*.

The pistil initial develops from the apex of the growing point which is located between the anther initials (pl. 5, *B*, *p*, and *I*, *p*). Development begins with the formation of a ridge, the silk initial, which partly encircles the tip of the growing point (pl. 5, *C*, *s*, and *D*, *s*). The ovule differentiates from the meristem which is partly enclosed by the developing silk initial (pl. 5, *C*, *ov*, and *E*, *ov*).

The margin of the silk initial grows more rapidly on one side than on the other (pl. 5, *E*, *s*). Soon two distinct points appear (pl. 5, *F* and *G*) which continue to elongate (pl. 5, *G* and *K*) and which finally result in the biparted tip of the mature silk (pl. 6, *E*). Unequal growth rates of the margins of the silk initial result in the development of a tubelike structure, partly enclosing the ovule (pl. 5, *E*, *ov*). The opening above the ovule gradually closes and becomes the stylar canal (pl. 5, *G*, *sc*; *H*, *sc;* and *K*, *sc*).

As the silk elongates it becomes covered with hairs, the structure of which has been described by Weatherwax (*16*). Hairs are just beginning to appear as fine points upon the silk in plate 5, *K*, *s*, and *L*, *s*, and they are shown, fully developed, with pollen grains germinating upon them, in plate 6, *E*, *F*, and *G*.

The ovary is shown in plate 6, *D*,*o*, with the silk attached and partly enclosed by the flowering glumes. At this stage of pistil development all of the external parts have differentiated but the pistil has not attained full size.

Silks begin to develop first at the base of the ear (pl. 6, *A*), and at later stages of ear development a marked contrast in the length of the silks at the tip and the base of the ear can be seen (pl. 6, *C*).

EXPLANATORY LEGEND FOR PLATE 7

A.—A staminate (left) and pistillate (right) spikelet from the tassel. ×20.

B.—Silk development in a pistillate spikelet from the tassel. ×28.

C.—Glumes removed from the pistillate spikelet to show the abortive lower flower. ×20.

D.—Staminate and pistillate spikelets of the tassel. ×15.

E.—A fully differentiated staminate and a pistillate spikelet of the tassel, the pistillate spikelet being sessile and the staminate spikelet pediceled. ×10.

F.—A tassel showing silks from pistillate spikelets at the base of the tassel. ×10.

fl 1, Upper flower; *fl* 2, lower flower; *p*, pistil; *s*, silk; *x*, upper flower of the staminate spikelet.

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An enlargement of a section of the ear at the same stage of development as the ear in plate 6, *A*, is shown in plate 6, *B*. The attachment of the spikelets, variation in the length of the silks, and the size of the stylar canal can all be seen in plate 6, *B*.

Very soon after pollen grains lodge upon the silk they germinate and the pollen tube grows down the hair into the silk (pl. 6, *F* and *G*). This process has been described by Miller (*8*) and Randolph (*9*).

DEVIATIONS FROM NORMAL FLOWER DEVELOPMENT

Two deviations from normal flower development will be illustrated and described. The first is the development of two fertile flowers in a pistillate spikelet and the second is the development of pistillate spikelets in the tassel. Illustrations for the first deviation were taken from Country Gentleman sweet corn and are shown in plate 5, *I*, *J*, *L*, and *M*. Illustrations for the second deviation were taken from Golden Cross Bantam and are shown in plate 7.

It will be recalled that in those types of corn that have only one fertile flower per spikelet, the sterile flower begins but does not complete its development. The sterile flower develops from the smaller of the two divisions of the growing point of the spikelet initial. Anther initials and the pistil initial of the sterile flower differentiate (pl. 5, *G*, fl_2) but do not complete their development (pl. 5, *H*, fl_2). The pistil of the fertile flower develops as has been described, but the anthers do not, so that in examining a spikelet of the ear of those types of corn having one fertile flower per spikelet, all that can be seen after the silk has begun to elongate are the empty glumes (pl. 5, *K*, *e*) and the silk of the fertile flower extending beyond them (pl. 5, *K*, *s*).

When two fertile flowers develop in a pistillate spikelet each flower goes through the same sequence of development that has been described, but the rates of development are different. The upper flower arising from the larger of the two divisions of the growing point develops more rapidly than the lower flower. This was also pointed out for the development of the two flowers of the spikelet in the tassel.

The differences in rates of development of the upper and lower flowers can be seen by comparing the development of the upper flower in plate 5, *I*, fl_1; *J*, fl_1; *L*, fl_1; and *M*, fl_1, with the development of the lower flower designated as fl_2 in the photographs just mentioned. While the upper flower develops first, the lower flower gradually overtakes the upper flower as the ear approaches maturity, so that at pollination, the silks of both flowers are approximately the same length.

Paired grains of corn result when two fertile flowers are produced per spikelet. The germ of the upper flower faces the tip of the ear and the germ of the lower flower faces the base of the ear, resulting in the kernels being placed back to back. With the development of two grains per spikelet the kernels may be crowded out of line so that there are irregular rows or a lack of rows as shown in Country Gentleman sweet corn.

Development of paired grains, according to Randolph (*11*), was first described in pod corn by Sturtevant (*14*), and Kempton (*6*) was the first to interpret correctly the development of paired grains as being the result of the development of two fertile flowers per spikelet. Weatherwax (*18*), Stratton (*13*), and others have also described the development of double kernels.

The development of pistillate spikelets in the tassel is an interesting deviation from normal development. Kempton (*6*) has pointed out that if there are pistillate flowers in the staminate inflorescence, it is the upper flower of the sessile spikelet that is pistillate and both of the flowers of the pediceled spikelet are staminate.

Spikelet differentiation and the first stages in the development of the flowers are the same in both the staminate and the pistillate spikelets of the tassel. The essential difference lies in the degree of development of the anthers and pistils. In the pistillate flowers both the anthers and the pistil differentiate but the pistil takes precedence in development; in the staminate flowers both the anthers and pistil differentiate but the anthers develop instead of the pistil.

In Golden Cross Bantam only one flower, the upper one, of the spikelets of the ear is fertile and the same is true of the pistillate spikelet produced in the tassel. Consistent with the development of the flowers of the ear of this type, the upper flower (pl. 7, *A*, fl_1, and *C*, fl_1) developed and the lower flower was abortive (pl. 7, *A*, fl_2, and *C*, fl_2).

Pistil differentiation and development were the same as previously described for the pistillate flower of the ear. The various stages in the development of the silk are shown in plate 7, *A*, *s*, to *F*, *s*, inclusive, and it can be seen that they are essentially the same as already described.

The development of the flowers of the staminate spikelet shows no deviation from normal development except that the pistil is a little further developed than in the tassels having only staminate spikelets. But the example shown in plate 7, *A*, *x*, should not, perhaps, be considered as typical because even in those plants that did not have pistillate spikelets, a considerably greater degree of pistil development was noted (pl. 3 *F*, *p*, and *H*, *p*) than would be expected in plants grown in the field. However, this is what should be expected of corn plants grown in the greenhouse under certain conditions of temperature and light.

SUMMARY

The developmental morphology of the tassel, the ear, and their parts were studied by dissecting them from the stem of the corn plant at the different stages of development. Photomicrographs were taken of the various stages.

From germination to the dehiscence of the anthers, the shoot of the corn plant passes through two stages of development. In the first stage leaves and axillary shoots are produced, and in the second stage the internodes of the stem elongate and the tassel, ear, and their parts differentiate and develop.

Tassel differentiation begins with the appearance of lateral projections, branch initials, which arise acropetally from the growing point of the central axis. The first initials to appear at the base of the central axis elongate to produce branches of the first order. Those above develop into two spikelet initials.

Branches of the second order arise as buds from the base and at the margins of the branches of the first order.

Differentiation of the ear also begins with the appearance of lateral projections which arise acropetally from the growing point.

In both the tassel and ear the spikelet-forming branch initials divide into two unequal parts to form the spikelet initials and in turn the spikelet initials divide into two unequal parts to form the flower initials.

In the tassel the spikelet developing from the larger division of the branch initial is pediceled and the spikelet from the smaller division is sessile. In the ear and tassel the larger initial begins the development of its parts ahead of the smaller initial.

Differences in the size of the flower initials in the tassel are correlated with a difference in the size and rate of development of the anthers. The larger (upper) flower initial is ahead of, and larger than, the anthers of the smaller (lower) flower initials, but as the flowers approach maturity the anthers of the lower flower are almost as large as the anthers of the upper flower.

In the ear the flower developing from the larger (upper) of the two flower initials becomes the fertile flower and the smaller (lower) flower initial the abortive flower in those types of corn that have only one fertile flower per spikelet. In those types that have two fertile flowers per spikelet the flower from the upper initial is larger and develops before the flower from the lower initial.

The empty glumes are the first of the spikelet parts to differentiate in the spikelets of the tassel and the ear.

Flower parts of the flower of the tassel and of the ear differentiate in the following order: Lemma and palea, anthers, and pistil. In the pistil the ovary, silk, and hairs on the silk develop in the order named.

Pistillate spikelets which develop in the tassel follow the same sequence in their development as the pistillate spikelets of the ear.

When two fertile flowers develop in the pistillate spikelet of the ear both flowers follow the normal sequence of development, but the upper flower develops ahead of the lower flower.

Because they do not terminate in apical spikelets the ear and tassel are indeterminate inflorescence.

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THE DIGESTIBILITY OF MATURE RANGE GRASSES AND RANGE MIXTURES FED ALONE AND WITH SUPPLEMENTS [1]

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INTRODUCTION

Bunch grasses have long been prized for their feeding value when mature. Where weather conditions permit their use, these grasses have been considered excellent winter feed for horses, cattle, and sheep.

The study herein reported was made to determine: (1) The digestibility of bluebunch fescue (*Festuca idahoensis* Elmer) and bluebunch wheatgrass (*Agropyron spicatum* (Pursh) Scribn. and Smith), which are widespread on Washington ranges (identified by Hitchcock (*8*)[3]); (2) the digestibility of a mixture of mature bluebunch wheatgrass and other forages occurring on a typical range; (3) the nutritive value of the top half of the bluebunch wheatgrass plant as compared to that of the whole plant cut about 1 inch above the crown of the bunch, in range mixtures composed largely of this grass; (4) the supplementary effect of ground Beldi barley and old-process linseed cake when fed with range mixtures composed largely of mature bluebunch wheatgrass; (5) the supplementary effect of linseed cake when fed at two levels; and (6) the performance of lambs and ewes fed similarly in metabolism experiments.

REVIEW OF LITERATURE

DIGESTION STUDIES

Kennedy and Dinsmore (*10*) reported one of the earliest studies on digestibility of range feeds. They fed green range forages cut in early summer to 3-year-old wethers for a period of 6 days. The green grasses were digested fairly well, but in most cases the other range forages were more completely digested.

Dinsmore and Kennedy (*4*) fed several native hays composed of a mixture of grasses and clover, but with a predominance of one grass, to 3-year-old wethers during a 5-day experimental period. All the nutrients were quite completely digested, and these hays were considered excellent fattening feeds.

Hart and his coworkers (*6*) studied the digestibility of range grass composed largely of soft chess (*Bromus hordeaceus L.*) gathered in September after the seed heads had shattered. They recorded a negative value for crude-protein digestibility. The wether lambs to which this grass was fed lost 3 to 5 pounds' weight in a 10-day feeding period, and their maximum consumption was a little under 1 pound daily, indicating the unpalatability of the grass. Watson and Horton

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[3] Italic numbers in parentheses refer to Literature Cited, p. 49.

(*20*) evaluated grass-hay quality on the basis of its digestible crude-protein content.

Knight, Hepner, and Nelson (*11*) concluded that the reason forages cure so well on the range and retain their nutritive value to a great extent is their high crude-fiber content. However, Hopper and Nesbitt (*9*) observed that a high crude-fiber content in range grasses and hays depressed their digestibility.

Christensen and Hopper (*3*) made a seasonal study of the digestibility of prairie hay fed to steers. These hays consisted of from 50 to 75 percent of western needlegrass or needle-and-thread grass (*Stipa comata* Trin. and Rupr.) and 25 to 50 percent of weeds and other grasses. The July cutting taken at the height of the growing season was most palatable, highest in nutrient content, and most digestible. The annual and biennial cuttings were quite similar in composition and digestibility. The April and October cuttings were similar in composition, but their digestibility was lower because of the old grass contained in the hay.

Armsby (*1*) concluded that there are comparatively small differences in the digestibility of identical feeds when consumed by the various species of ruminants unless the crude-fiber content is high, in which case cattle are superior to sheep in their ability to digest such feeds. Forbes and his coworkers (*5*) found that sheep digested all nutrients except crude fiber more efficiently than cattle in a ration containing good-quality roughage and concentrates, but cattle excelled in ability to digest crude fiber.

In digestion experiments carried on by Roberts (*15*), a native hay composed largely of western wheatgrass or bluestem (*Agropyron smithii* Rydb.) proved to be a more valuable feed than timothy hay.

Headden (*7*) reported that the native hay of Colorado was considably superior to timothy in its digestible crude-protein content, but contained about the same amount of digestible dry matter. McCreary (*12*) concluded that after range grasses mature in the fall, they have a feeding value that appears to be little better than that of oat straw.

CONCENTRATE SUPPLEMENTS TO BUNCH GRASS

Mature range grasses and hays have wide nutritive ratios, according to Hopper and Nesbitt (*9*), and require a high-protein feed to supplement them so that sufficient protein will be available to meet the requirements of production, reproduction, and growth.

Anderson [4] contends that a winter supplement is necessary for ewes wintered on mature bunch grass unless they go into the winter in high condition, for without it many are unable to withstand the severe storms that often occur in the Northwest. He observed that cottonseed cake was superior to corn or beet-pulp pellets as a supplement to a bluebunch fescue range near Bozeman, Mont. However, Smith (*17*) found little difference between corn and cottonseed cake as supplements to the short-grass range near Miles City, Mont. This was probably due to the higher crude-protein content of the grass at Miles City. Maynard (*13*) reported an experiment in Utah where one-third of a pound of cottonseed cake was fed daily with wild hay, which is low in protein, to old range ewes. Lambs were produced

[4] ANDERSON, I. M. C. WINTERING EWES ON THE RANGE. U. S. Dept. Agr., Ext. Anim. Husbandman Ser. 40: 12-15. 1935. [Mimeographed.] See p. 15.

that weighed 6 pounds per head more by June 1 than lambs from ewes fed one-third of a pound of shelled corn with the same kind and amount of hay.

CHARACTERISTICS OF GRASSES FED

The Forest Service (*19, pp. G 6*) reported that bluebunch fescue is quite palatable in the spring when it is young and tender and again in the fall when cured, but in the summer when it becomes rather tough and dry, it is not so readily grazed.

Observations of the Forest Service (*19, pp. G 58*) indicated that bluebunch wheatgrass is palatable both green and cured, and that each plant has a large amount of tender foliage. It is found on early and late ranges where other palatable grasses are apt to be scarce, but, according to Sampson (*16*), it does not produce a heavy stand. McCreary (*12*) analyzed several range grasses and found bluebunch wheatgrass to be lowest in crude protein and comparatively high in ash and crude fiber.

EXPERIMENTAL ANIMALS, MATERIALS, AND METHODS

Range lambs of mixed breeding were used as experimental animals in 1930 and 1933, and in 1932 purebred Rambouillet lambs and pregnant Rambouillet ewes were fed. A detailed report of the method of experimentation, as well as of the metabolism crates used, has been previously presented by Sotola (*18*) of this station.

All sheep used in this study were given all the forage they would consume without waste. There was a little refuse feed in a few cases, but this was analyzed and deducted. A prescribed amount of concentrate was fed.

The pure stands of bunch grass were gathered on a south slope near Pullman, Wash. on a silt loam type of soil. The range mixtures were collected on a typical range of the semiarid hills of central Washington near Prosser on a sandy loam to a silt loam soil. The range mixtures gathered in 1930, 1932, and 1933 consisted of 90, 93, and 97 percent, respectively, of bluebunch wheatgrass. In 1930 the remainder consisted of about equal parts of the tender tips of common sagebrush *Artemisia trudentata* Nutt.), Russian-thistle *Salsola pestifer* A. Nels.), and Jim Hill mustard *Sisynbrium altissimum* L.). In 1932, 5 percent of sage tips and 1 percent of Russian-thistle and Jim Hill mustard were added to the bunch grass. In 1933 only 3 percent of sage tips were added as there was little other range forage available that was palatable to sheep, and the sage tips were not as luxuriant as in other years.

All the grasses and other forages studied were collected from late October to the first part of December. The seeds of these grasses were almost completely shattered out when collected.

The grass in the range mixture collected in 1930 consisted of the whole plant cut about 1 inch above the crown of the bunch with a hand scythe. As the digestibility of this mixture was very low, only the top half of the grass plant was collected in 1932 and 1933 to see if this portion would prove more digestible. This method of cutting more nearly simulates the grazing of sheep having access to sufficient range.

Coarsely ground Beldi barley (*Hordeum vulgare* L.) and pea-size old-process linseed cake were fed as supplements to the range mixtures. The concentrates were about average in quality, and were fed mixed with the chopped forage.

Analytical methods as reported by the Association of Official Agricultural Chemists (*2*) were used.

CHEMICAL COMPOSITION OF FEEDS

The chemical analyses of the grasses, range mixtures, concentrates, and rations, including range mixtures and concentrates, are summarized in table 1. The grasses and range mixtures were quite high in crude fiber and low in crude protein. These tendencies were not so great in bluebunch fescue.

Bluebunch fescue contained more crude protein and ash and less crude fiber than bluebunch wheatgrass. The range mixture collected in 1933 contained less ash and crude fat and more crude fiber than that collected in 1932. In both of these collections, only the top half of the bunch was used. Most of the growing season of March, April, May, and June of 1933 was cooler and the precipitation greater than in the similar period of 1932. This seasonal variation in nutrient content is in accord with the results of Woodman and his coworkers (*21*) in their study of pasture grasses. The range mixture collected in 1932 was particularly high in crude fat, and the grass included in the range mixture had the golden color that stockmen associate with high feeding value.

TABLE 1.—*Chemical composition of range grasses, range mixtures, concentrates, and mixed rations*

Feed	Year forage collected	Part of plant collected	Experiment No.	Dry matter	Ash	Crude protein (N×6.25)	Carbohydrates		Crude fat
							Crude fiber	Nitrogen-free extract	
				Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Bluebunch fescue	1930	Whole plant	2	87.70	14.60	4.62	27.18	38.65	2.65
Bluebunch wheatgrass	1930	do	1	88.74	10.14	2.94	33.37	39.61	2.68
Range mixture	1930	do	3	88.06	7.96	3.73	29.36	43.54	3.47
Do	1932	Top half	4	89.91	12.88	2.49	31.52	38.29	4.73
Do	1932	do	5, 6	90.70	12.82	2.44	31.80	38.87	4.77
Do	1932	do	7	91.17	12.89	2.45	31.74	39.29	4.80
Do	1932	do	9, 10	91.29	12.91	2.45	32.00	39.13	4.80
Do	1933	do	11	86.99	6.62	2.12	34.50	41.87	1.88
Do	1933	do	12	88.12	6.84	2.09	34.75	42.52	1.92
Do	1933	do	13	89.09	6.93	2.06	36.87	41.79	1.44
Do	1933	do	14	88.17	6.51	2.43	36.89	40.94	1.40
Barley			5	90.30	2.54	8.59	5.60	72.02	1.55
Do			7	89.29	2.57	10.34	5.54	69.31	1.53
Do			12	87.44	2.88	9.66	4.70	68.70	1.50
Linseed cake			6	92.40	7.35	32.23	7.81	39.46	5.55
Do			9, 10	92.27	8.29	35.60	7.80	35.04	5.54
Do			13	89.80	5.81	33.73	8.12	36.75	5.39
Do			14	89.50	5.79	34.44	8.21	35.68	5.38
Range mixture plus—									
Barley	1932		5	88.49	8.45	4.92	20.39	51.51	3.22
Linseed cake	1932		6	91.20	10.97	12.48	23.76	38.96	5.03
Barley	1932		7	90.05	8.44	5.82	21.81	50.73	3.25
Linseed cake	1932		9	91.34	12.20	7.50	28.35	38.30	4.99
Do	1932		10	91.61	11.38	13.42	23.99	37.78	5.04
Barley	1933		12	87.86	5.35	4.94	23.43	52.38	1.76
Linseed cake	1933		13	89.18	6.80	5.86	33.42	41.19	1.91
Do	1933		14	88.49	6.34	10.06	30.05	39.69	2.35

RESULTS OF THE DIGESTION STUDIES

FEED CONSUMPTION

Table 2 shows the amount of nutrients consumed by the lambs and ewes. Because of the difference in size of the experimental sheep in the 3 years of this study, the feed consumption was computed to the basis of 100 pounds of body weight. The lambs fed range mixture and the high level of linseed cake in experiment 14 consumed the largest amount of dry matter, crude protein, crude fiber, and nitrogen-free extract. The addition of linseed cake to the range mixtures fed in 1932 and 1933 considerably increased the palatability of these forages. The addition of barley did not have as much effect in this respect. The second largest amount of dry matter consumed was from the range mixture collected in 1930, and more forage dry matter was consumed than in any other ration. The lowest dry-matter consumption by lambs was from the range mixture collected in 1933, which was quite high in crude fiber and low in crude fat. Although there was a slight difference in forages other than grass in the range mixture collected in 1930 and 1933, and the growing and maturing seasons were somewhat different, the considerable variation in the consumption of these forages would indicate that the top half of the

TABLE 2.—*Relative amount of feed and nutrients consumed daily by lambs and ewes fed bunch grasses and range mixtures alone and with concentrates*

[Computed to 100 pounds (45.36 kg.) body weight]

Feed, level, and year forage collected	Experiment No.	Sheep	Kind of sheep	Average weight of sheep	Kind of feed		Feed and nutrients consumed per head daily, computed to 100 pounds body weight						
					Forage	Concentrate	Feed as fed	Dry matter	Ash	Crude protein (N×6.25)	Carbohydrates: Crude fiber	Carbohydrates: Nitrogen-free extract	Crude fat
		No.		*Kg.*	*Pct.*	*Pct.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Bluebunch fescue—pure stand:													
1930 [1]	2	6	Lambs	24.7	100.00	0.00	901.5	790.6	131.6	41.6	245.0	348.5	23.9
Bluebunch wheatgrass—pure stand:													
1930 [1]	1	6	do	24.2	100.00	.00	780.2	692.5	79.1	22.9	260.4	309.2	20.9
Range mixture:													
1930 [1]	3	6	do	24.4	100.00	.00	1,005.8	885.7	80.1	37.5	295.3	437.9	34.9
1933 [2]	11	6	do	29.6	100.00	.00	331.5	288.4	21.9	7.0	114.4	138.9	6.2
1932 [2]	4	6	Ewes [3]	65.0	100.00	.00	323.5	290.8	41.7	8.0	102.0	123.8	15.3
Range mixture plus barley—high level:													
1932	7	3	Lambs	45.7	55.87	44.13	589.5	530.8	49.8	34.3	128.6	298.9	19.2
1933	12	6	do	29.2	62.33	37.67	724.5	636.5	38.8	35.8	169.7	379.4	12.8
1932	5	3	Ewes [3]	69.1	55.50	44.50	486.6	430.6	41.1	23.9	99.2	250.7	15.7
Range mixture plus linseed cake—low level:													
1932	9	3	Lambs	45.7	85.29	14.71	607.2	554.6	74.1	45.5	172.1	232.6	30.3
1933	13	6	do	28.3	87.98	12.02	801.4	714.7	54.5	47.0	267.8	330.1	15.3
Range mixture plus linseed cake—high level:													
1932	10	3	do	51.2	66.90	33.10	744.9	682.4	84.8	100.0	178.7	281.4	37.5
1933	14	6	do	29.9	76.16	23.84	1,118.7	989.9	70.9	112.5	336.2	444.0	26.3
1932	6	3	Ewes [3]	62.2	66.21	33.79	713.7	650.9	78.3	89.1	169.6	278.0	35.9

[1] Grass cut about 1 inch above the crown of the bunch.
[2] Bluebunch wheatgrass in range mixture cut about halfway from crown to top of bunch.
[3] 2 years old.

bluebunch wheatgrass plant was less palatable than the whole plant. When no concentrate was fed, the consumption of bunchgrass and range mixtures was inadequate for maintenance except in experiment 3 with the range mixture collected in 1930. But according to Armsby (*1*) the amount of feed consumed has little effect on its digestibility as long as the kind of feed consumed remains the same and the experimental animals are similar.

COEFFICIENTS OF APPARENT DIGESTIBILITY

The average coefficients of apparent digestibility obtained with lambs and ewes for the organic nutrients of the rations fed are summarized in table 3. Six lambs were used except in 1932, when three lambs were fed one ration and the other three another ration as indicated in table 3.

TABLE 3.—*Coefficients of digestibility of bunch grasses and range mixtures fed alone and supplemented with concentrates to lambs and ewes*

[Standard deviations [1] are reported only where six sheep were fed]

Feed, level, and year forage was collected	Experiment No.	Sheep	Kind of sheep	Coefficients of digestibility				
				Dry matter	Crude protein (N×6.25)	Carbohydrates		Crude fat
						Crude fiber	Nitrogen-free extract	
Bluebunch fescue—pure stand:		*Number*		*Percent*	*Percent*	*Percent*	*Percent*	*Per cent*
1930 [2]	2	6	Lambs	41.5±2.08	21.2±5.00	55.3±7.12	49.8±2.23	33.5±2.26
Bluebunch wheatgrass—pure stand:								
1930 [3]	1	6	do	36.0±2.76		50.7±2.66	38.8±3.06	32.0±2.00
Range mixture:								
1930 [3]	3	6	do	47.5±2.26	17.2±6.43	55.7±2.66	49.8±2.23	44.7±2.50
1933 [3]	11	6	do	51.0±8.03		67.0±8.54	52.6±8.79	49.4±4.16
1932 [3]	4	6	Ewes [4]	27.8±5.31		43.5±4.68	33.5±5.79	27.2±6.85
Range mixture plus barley—high level:								
1932	7	3	Lambs	58.7	47.0	57.3	71.0	47.0
1933	12	6	do	59.2±3.31	34.4±6.19	57.3±6.06	68.2±2.64	58.7±4.72
1932	5	3	Ewes [4]	47.3	33.0	37.0	60.3	45.3
Range mixture plus linseed cake—low level:								
1932	9	3	Lambs	40.3	37.0	57.0	41.0	69.7
1933	13	6	do	52.3±4.32	38.0±7.35	65.5±5.24	51.5±3.98	58.7±5.13
Range mixture plus linseed cake—high level:								
1932	10	3	do	53.0	67.7	56.3	58.3	66.7
1933	14	6	do	56.0±2.76	62.3±3.39	67.0±3.41	54.8±2.48	65.8±4.17
1932	6	3	Ewes [4]	45.3	63.3	53.0	52.3	52.0

[1] The formula used in computing the standard deviation was $\sigma = \sqrt{\frac{\Sigma d^2}{(n-1)}}$

[2] Grass cut about 1 inch above the crown of the bunch.
[3] Grass in range mixture cut about halfway from crown to top of bunch.
[4] 2 years old.

All the nutrients of the range mixture fed in 1930, which consisted largely of bluebunch wheatgrass, were more completely digested than those in the pure stand of this grass. The digestibility of the dry matter of the mature grasses and the range mixtures was quite low, with the exception of the range mixture collected in 1933. The consumption of the 1933 range mixture was low, but, according to Armsby (*1*), this should make little difference in its digestibility. The addition of barley and linseed cake to the range mixture increased the

digestibility of dry matter of the mixed ration in all cases as compared to that of the range mixture fed alone.

More crude protein was excreted than was consumed by lambs fed the pure stand of bluebunch wheatgrass and the 1933 range mixture, and by ewes fed the 1932 range mixture. According to Mitchell *(14)* the greater the concentration of indigestible, nonnitrogenous material in a ration, the greater the fecal excretion of metabolic nitrogen. The addition to range mixtures of barley and of linseed cake (particularly the larger amount) materially increased the digestibility of crude protein and crude fat. No similar increase was noted in the digestibility of crude fiber, and only when barley was added to the range mixture was there a significant increase in the digestibility of nitrogen-free extract.

The coefficients of digestibility obtained with lambs were in all cases higher than with ewes similarly fed. The ewes fed in these experiments were well along in their pregnancy period.

The range mixtures fed with linseed cake at the low level were in general less completely digested than when fed with barley at the high level, which was about three times as much concentrate. However, only the dry matter and nitrogen-free extract of the latter ration were more completely digested than that of the range mixtures and the larger amount of linseed cake. About one-half pound of concentrate per 100 pounds of live weight was fed at the high level.

TOTAL DIGESTIBLE NUTRIENT RATING

Table 4 presents a summary of the digestible nutrients in the rations fed and a rating of the rations based on the total digestible nutrients contained.

TABLE 4.—*The digestible nutrients in bunch grasses and range mixtures fed alone and with concentrates; rating based on total digestible nutrients*

Feed level, and year forage was collected	Experiment No.	Kind of sheep	Total dry matter in ration	Digestible nutrients					Nutritive ratio, 1 to—	Rating on basis of total digestible nutrient
				Crude protein (N×6.25)	Carbohydrates		Crude fat	Total		
					Crude fiber	Nitrogen-free extract				
Bluebunch fescue—pure stand:			*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*		*Percent*
1930	2	Lambs	87.70	0.98	15.03	19.32	0.89	37.33	37.09	88.67
Bluebunch wheatgrass—pure stand:										
1930	1	do	88.74	.....	16.92	15.37	.86	34.23	.....	81.31
Range mixture:										
1930	3	do	88.06	.64	16.29	21.68	1.55	42.10	64.78	100.00
1933	11	do	86.99	.....	23.12	22.02	.93	47.23	.....	112.18
1932	4	Ewes [1]	89.91	.....	13.71	12.83	1.29	29.44	.....	69.93
Range mixture plus barley—high level:										
1932	7	Lambs	90.05	2.74	12.50	36.02	1.53	54.70	18.96	129.93
1933	12	do	87.86	1.69	13.42	35.72	1.03	53.15	30.45	126.25
1932	5	Ewes [1]	88.49	1.62	7.54	31.06	1.46	43.50	25.85	103.32
Range mixture plus linseed cake—low level:										
1932	9	Lambs	91.34	2.78	16.16	15.70	3.48	42.48	14.28	100.90
1933	13	do	89.18	2.23	21.89	21.21	1.12	47.85	20.46	113.66
Range mixture plus linseed cake—high level:										
1932	10	do	91.61	9.08	13.51	22.02	3.36	52.17	4.74	123.92
1933	14	do	88.49	6.27	20.13	21.75	1.55	51.64	7.24	122.66
1932	6	Ewes [1]	91.20	7.90	12.59	20.38	2.62	46.77	4.92	111.09

[1] 2 years old.

When the total digestible nutrients of the range mixture fed in 1930 were figured at 100 percent, the range mixture and barley combinations fed to lambs in 1932 and 1933 were highest, with ratings of 129.93 percent and 126.25 percent, respectively. The range mixture fed to ewes in 1932 rated only about one-half as high, or 69.93 percent. These ewes consumed a small amount of forage and its digestibility was quite low. All rations containing some concentrate rated 100 percent or higher.

The nutritive ratios of the grasses and of range mixtures fed alone were quite wide in cases where there were positive coefficients of digestibility of crude protein. The nutritive ratios were very narrow only when the high level of linseed cake was fed with the range mixtures.

NITROGEN BALANCES

The nitrogen consumption and retention for lambs and ewes during the 10-day period on the different feeds are reported in table 5. The addition of the larger amount of linseed cake to the range mixtures more than doubled the nitrogen intake over that from range mixtures and barley. The smallest nitrogen consumption from the rations containing no concentrate was 7.28 gm. as compared with 118.16 gm., the smallest amount consumed when the linseed cake was fed at the high level. More than 16 times as much nitrogen was consumed in the latter ration. The nitrogen consumption in the former case was very low because of the small amount of feed eaten and its low nitrogen content.

TABLE 5.—*Summary of nitrogen balances for lambs and ewes fed bunch grasses and range mixtures alone and supplemented with concentrates; 10-day period*

[Standard deviations given only for experiments with 6 sheep]

Feed, level, and year forage was collected	Experiment no.	Sheep	Kind of sheep	Nitrogen consumed	Nitrogen voided in—			Nitrogen balance	Intake stored
					Feces	Urine	Total		
Bluebunch fescue—pure stand:		*Number*		*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*
1930 [1]	2	6	Lambs	36.37±5.50	28.49±3.98	15.54±1.97	44.02±4.87	−7.65	
Bluebunch wheatgrass—pure stand:									
1930 [1]	1	6	do	19.50±3.68	22.72±3.42	13.72±1.81	36.44±3.76	−16.94	
Range mixture:									
1930 [1]	3	6	do	32.38±4.55	26.75±4.80	13.56±2.43	40.31±6.12	−7.93	
1933 [2]	11	6	do	7.28±2.63	12.57±4.15	20.90±2.65	33.47±3.09	−26.19	
1932 [2]	4	6	Ewes [3]	18.60±5.30	28.20±6.46	43.46±7.96	71.66±10.09	−53.06	
Range mixture plus barley—high level:									
1932	7	3	Lambs	55.59	29.53	19.26	48.81	+6.78	12.20
1933	12	6	do	36.73±1.69	24.14±2.29	15.33±5.78	39.47±2.70	−2.74	
1932	5	3	Ewes [3]	59.13	40.01	28.93	68.94	−9.81	
Range mixture plus linseed cake—low level:									
1932	9	3	Lambs	70.68	44.30	42.09	86.39	−15.71	
1933	13	6	do	46.87±1.71	29.04±4.36	23.53±2.20	52.57±5.69	−5.70	
Range mixture plus linseed cake—high level:									
1932	10	3	do	178.43	58.11	85.94	144.05	+34.38	19.27
1933	14	6	do	118.16±2.65	44.47±4.62	43.41±6.60	87.88±5.86	+30.28	25.60±5.27
1932	6	3	Ewes [3]	195.01	71.70	123.63	195.33	−.32	

[1] Grass cut about 1 inch above crown of bunch.
[2] Bluebunch wheatgrass in range mixture cut about halfway from crown to top of bunch.
[3] 2 years old.

There was no nitrogen retention from bunch grasses or range mixtures fed with no concentrate supplement, and in only one case when barley was fed with a range mixture. However, all the lambs fed the higher level of linseed cake stored about one-fifth to one-fourth of the nitrogen consumed, and the nitrogen consumed and voided by the ewes on this feed just about balanced. Linseed cake was decidedly superior to barley as a supplement from the standpoint of nitrogen retention.

SUMMARY AND CONCLUSIONS

Bluebunch fescue (*Festuca idahoensis*, Elmer) cut in the late fall of 1930 about 1 inch above the crown of the bunch contained more crude protein, less crude fiber, and more total digestible nutrients when fed to lambs than bluebunch wheatgrass (*Agropyron spicatum* (Pursh) Scrbn. and Smith). Coefficients of digestibility of crude protein were positive for bluebunch fescue and negative for bluebunch wheatgrass, although lambs fed both of these grasses registered negative nitrogen balances. The bluebunch fescue was more palatable than the bluebunch wheatgrass.

A range mixture consisting of bluebunch wheatgrass with 10 percent of other forages collected in the late fall of 1930 contained more crude protein, nitrogen, free extract, and crude fat, less crude fiber and ash, and was more digestible and palatable than the same grass fed alone. The bluebunch wheatgrass of this mixture was also cut about 1 inch above the crown of the bunch. The range mixture of 1930 contained less crude fiber, more crude fat, more crude protein of higher digestibility, and was considerably more palatable than the range mixture collected in 1933, which contained only the top half of the bluebunch wheatgrass. The range mixture collected in 1932, similar in physical composition to that of 1933, but containing considerably more crude fat and ash, a little less crude fiber and nitrogen-free extract, and about the same amount of total digestible nutrients, seemed to be more palatable than that collected in 1933 when fed in combination with ground Beldi barley and old-process linseed cake. These comparisons would indicate that the larger proportion of basal leaves in the 1930 range mixture more than compensated for the lower part of the stems included as compared to the 1932 and 1933 range mixture. The range mixture collected in 1930 furnished enough digestible nutrients to meet the maintenance requirements of the lambs to which it was fed, but the other range mixtures, as well as the pure stands of bluebunch fescue and bluebunch wheatgrass, failed to meet these requirements.

When approximately one-half pound of linseed cake per 100 pounds of live weight was fed with range mixtures to lambs and pregnant ewes, the mixed rations were more palatable and digestible than the same range mixtures fed alone. This combination was also more palatable and contained more digestible crude protein, crude fiber, and crude fat than a similar amount of ground barley fed with the same range mixture, but the barley combination contained slightly more total digestible nutrients because of a greater content and digestibility of nitrogen-free extract.

A ration consisting of approximately one-sixth of a pound of linseed cake per 100 pounds live weight of lambs and a range mixture collected in 1933, was more palatable and contained more total digestible

nutrients than a similar range mixture fed alone. This ration was also more palatable than approximately one-half pound of barley and a similar range mixture, but did not contain as much total digestible nutrients as the latter ration. Approximately one-half pound of linseed cake and range mixture was more palatable and digestible than the smaller amount of linseed cake and range mixture, and the former ration was the only one of this study that had a very narrow nutritive ratio.

Pregnant ewes were less efficient than lambs in digesting range mixtures with or without the addition of barley. They more closely approached lambs in efficiency of digestion when fed linseed cake as a supplement to a similar range mixture.

It would seem from this study that mature bluebunch fescue and bluebunch wheatgrass collected in late fall have been overrated as feed for sheep.

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RELATION BETWEEN CAROTENOID CONTENT AND NUMBER OF GENES PER CELL IN DIPLOID AND TETRAPLOID CORN [1]

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INTRODUCTION

The evaluation of induced chromosome doubling as a method of plant breeding requires more information on the specific effects of chromosome doubling than is available at the present time. It is important that this information be procured from comparisons of related diploid and tetraploid strains of known origin and similar genetic constitution. Such strains have not been generally available because of the infrequency with which chromosome doubling occurs spontaneously and because of the lack of methods for the experimental production of autotetraploids generally applicable to crop plants. But with the development of the heat-treatment technique for the experimental doubling of chromosome numbers (*10*) [3] comparable diploid and tetraploid strains of corn (*Zea mays* L.) and other plants have been made available for study.

The effect of chromosome doubling on the carotenoid pigments in corn is of special interest because of the vitamin A potency of two of these pigments. The investigation reported in this paper was undertaken to determine the relation between carotenoid content, cell volume, and gene number in comparable strains of diploid and tetraploid corn and to determine the carotenoid content of examples of commercial varieties, inbred strains, and hybrids of ordinary diploid corn. A preliminary report of these studies has already been published (*12*).

The carotenoids of the corn kernel are located in the endosperm tissue, which is relatively homogeneous in cellular organization and is thus favorable material for a study of cell-volume relations. In corn of the ordinary diploid sort the chromosomes and genes are present in triplicate, while in the derived tetraploid there are six sets of chromosomes and genes. Since the character in question is definitely localized in the endosperm tissue, a favorable opportunity is presented for a study of the effect of gene number on the degree of development of the yellow pigment.

Yellow corn meal contains the carotenoid pigments beta-carotene and cryptoxanthin, precursors of vitamin A, and zeaxanthin, which

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[2] The carotenoid analyses were made by R. G. Hart in the dairy chemistry laboratory, New York State College of Agriculture.

[3] Italic numbers in parentheses refer to Literature Cited, p. 64.

has no relation to vitamin A activity (*7*). In animal experiments, Steenbock and Boutwell (*14*) demonstrated the vitamin A potency of yellow corn and the lack of potency of white corn. Later, Mangelsdorf and Fraps (*8*) and Johnson and Miller (*3, 4*) found a direct quantitative relationship between the amount of vitamin A potency and the number of dominant genes for yellow in comparable samples of corn.

Studies on the chemical composition of diploids and their autotetraploid derivatives have been made in relatively few plants. The relation between chromosome number and vitamin C content in apples was studied by Crane and Zilva (*1*), who found that triploid varieties had more of the vitamin than did the unrelated diploid varieties that were examined. Autotetraploid tomatoes also contain more vitamin C than the parental diploids, according to Sansome and Zilva (*13*). Chemical analyses of comparable diploid and autotetraploid strains of tomatoes and petunias were made by Kostoff and Axamitnaja (*5*), who reported that the tetraploid tomatoes had more nitrogen and water but less cellulose and ash than the parental diploid strain; but in *Petunia* the chemical composition of the diploid and tetraploid was essentially the same. The results with tomatoes should be interpreted in the light of the fact that tetrapoid tomatoes, because of their reduced fertility, have smaller fruits than the diploids.

PREPARATION OF MATERIALS

The strains of diploid and tetraploid yellow corn selected for comparison were derived from three inbred lines of pure yellow corn, Webber Dent 2312, Illinois A–2311, and Luces Favorite W36–1. The Webber Dent inbred was crossed with Illinois A, and the F_1 hybrid was crossed in turn with the Luces Favorite inbred. Chromosome doubling was induced in this three-way hybrid by the heat-treatment technique (*10*), and the resulting tetraploid plants, several in number, were mass-pollinated for two generations to provide adequate material for analysis. A comparable diploid strain was developed from the same source by mass-pollinating for two generations the diploid sister plants of the induced tetraploid individuals. The two strains were designated Diploid Yellow and Tetraploid Yellow, respectively. A second tetraploid yellow strain, designated Tetraploid Yellow B, was also analyzed for total carotenoid content. This strain originated from a cross between an inbred white flint corn and the Webber Dent 2312 inbred, followed by three generations of selective breeding for the yellow character. Comparable diploid and tetraploid strains of white corn, derived from inbred lines of white Argentine Flint and Spanish Flint and here designated Diploid White and Tetraploid White, were also analyzed for carotenoid content. In addition, separate analyses were made of a number of diploid inbred lines, including those from which the above-mentioned strains were derived.

In addition to these analyses of tetraploid and diploid corn, the carotenoids were determined in examples of various commercial varieties, inbred lines, and their hybrids, including a commercial double-cross hybrid grown extensively for grain and fodder in New York.[4]

[4] The hybrid, W29–3, and the parent lines, W36–1, W36–2, W36–3, and W36–4, were produced by Dr. R. G. Wiggans, of the Department of Plant Breeding, New York (Cornell) Agricultural Experiment Station, and were furnished by him for these studies.

The comparison of cell-volume relations in the endosperm tissue of the diploid and tetraploid corn was based upon a study of morphologically mature kernels selected from the pure yellow strains, which were analyzed for carotenoid content. Microtome sections were prepared in the usual manner from the desired portions of kernels that had been fixed in a weak Flemming's solution. To facilitate the preparation of the sections, the reserve starch in the endosperm was partly removed by soaking the seeds in water prior to fixation. Cell volumes were computed from measurements taken from camera-lucida sketches drawn at a magnification of 440 diameters.

ANALYTICAL METHOD

For the fractionation and determination of the carotenoids in the corn meal, the following simplified method, based upon the procedure of Kuhn and Brockmann (*6*), was developed. The meal was prepared from entire kernels, including the embryo and pericarp in addition to the endosperm. Since the endosperm comprised the bulk of the meal and the proportions of the different parts were very similar in the diploid and tetraploid stocks that were analyzed, the presence of the germ and pericarp was disregarded in comparing the relative amounts of carotenoid present in the samples. Samples were prepared for analysis by selecting at random 10 ears of corn from each strain. Corn meal was produced from the air-dried, shelled grain by grinding 50-gm. samples for 5 minutes in a Wiley mill equipped with a sieve of 1-mm. mesh. The meal was dried in a desiccator over phosphorus pentoxide (P_2O_5). Four to eight samples of 2.5 gm. each were thoroughly mixed with 50 ml. of anhydrous methyl alcohol (distilled from lime) and allowed to stand in glass-stoppered flasks for 18 hours. The methyl alcohol extract was filtered through sintered glass, the filter and precipitate were washed with small portions of methyl alcohol, and the volume was reduced by evaporation under reduced pressure and then made up to exactly 50 ml. with additional methyl alcohol. The total pigment in the methyl alcohol was determined in a photoelectric colorimeter with Corning glass filters 585 and 428. The construction, calibration, and use of this colorimeter has been described by Hand and Sharp (*1a*). The extract was saponified for 2 hours at 50° C. after 5 ml. of a 5-percent solution of potassium hydroxide (KOH) in methyl alcohol had been added. The solution was cooled, 6.0 ml. of water added, and the mixture shaken for 15 seconds in a separatory funnel. The active fraction, containing beta-carotene and cryptoxanthin, was extracted with from 4 to 8 successive 25-ml. portions of petroleum ether.[5] The petroleum ether extracts were combined and evaporated to exactly 50 ml. under reduced pressure, and the absorption coefficient was determined in the colorimeter. In a similar manner the inactive zeaxanthin was extracted with petroleum ether after the water content of the methyl alcohol had been increased to 40 percent, and the absorption coefficient in petroleum ether was determined.

Calculations of carotenoid were made from a calibration curve for the absorption coefficient of pure beta-carotene plotted against

[5] Separate determination of beta-carotene and cryptoxanthin can be made by extracting the beta-carotene from 99-percent methyl alcohol and the cryptoxanthin from 90-percent methyl alcohol, but were not made in these studies because of the relatively small amount of beta-carotene in corn meal.

milligrams of beta-carotene per liter of petroleum ether (fig. 1). In these calculations, it was assumed that cryptoxanthin and zeaxanthin had the same absorption as beta-carotene with glass filters 585 and 428 and that the light absorption was the same in methyl alcohol as in petroleum ether. Since these assumptions are not exactly correct, an error was introduced with respect to the absolute amounts of carotenoid, but not with respect to the relative values for the different

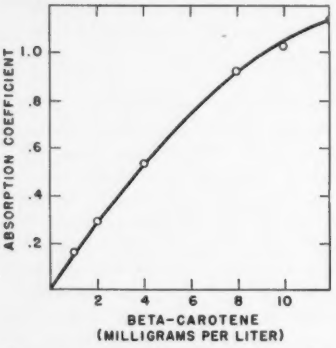


FIGURE 1.—Light absorption by beta-carotene in petroleum ether. Absorption coefficient, $\log_{10} \frac{I_o}{I}$ for 1 cm., filters 585 and 428.

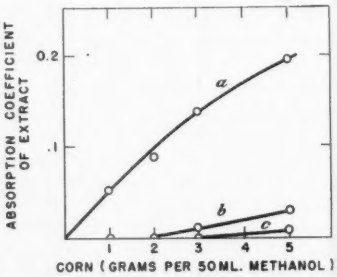


FIGURE 2.—Repeated extractions of corn samples by methanol: *a*, First extraction; *b*, second extraction; *c*, third extraction.

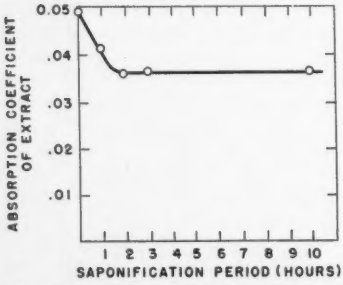


FIGURE 3.—Effect of saponification time on the extraction of carotenoids by petroleum ether from 85-percent methanol.

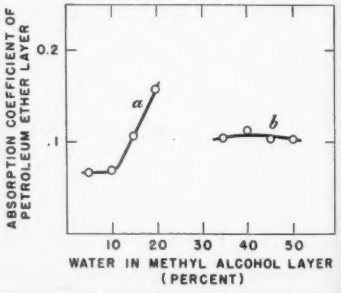


FIGURE 4.—Effect of water content on the fractionation of carotenoids: *a*, Active fraction; *b*, inactive fraction.

strains of corn. In practice the calculation was further simplified so that the number of milligrams of carotenoid per gram sample was obtained by multiplying the absorption coefficient by the factor 0.155. This approximate method neglects the deviation from linearity up to 5 mg. per liter (fig. 1). The chief advantage in using the factor is that the calculated values for carotenoid content can be readily changed to the experimental values for the absorption coefficients of 50-ml. extracts from 2.5-gm. samples of corn.

Experiments made in the elaboration of the method are summarized in the accompanying figures. In figure 2 it can be seen that practically all of the carotenoid is removed by a single extraction with 50 ml. of methyl alcohol if the sample is not larger than 2.5 gm. Figure 3 shows that the saponification of the xanthophyll esters is complete after 2 hours at 50° C. If the saponification is incomplete, the xanthophyll esters are extracted with the beta-carotene and cryptoxanthin and the values for the active fraction are erroneously high. If the saponification is carried on too long, e. g., for 10 hours, extraction of the zeaxanthin fraction by petroleum ether is very much more difficult. Figure 4 shows that a sharp separation of the active and inactive fractions is obtained with 10 percent of water in the methyl alcohol. If more water is used for the first extraction, some of the zeaxanthin is forced into the petroleum ether. The amount of water needed for the optimum extraction of the inactive fraction may vary from 30 to 50 percent.

Some idea of the accuracy of the method can be obtained from a series of 17 analyses of the same strain of corn for which the average deviation from the mean was only ±4.2 percent. By reducing the number of steps in the procedure the chances for the loss or destruction of carotenoids were reduced. The results reported herein agree with typical values for the carotenoids in a sample of Italian corn reported by Kuhn and Grundmann (*7*) (beta-carotene, 0.0007 mg.; cryptoxanthin, 0.0046 mg.; and zeaxanthin, 0.0127 mg. per gram) but are considerably higher than those of Johnson and Miller (*3*, *4*).

RESULTS OF CAROTENOID ANALYSES

The results of the carotenoid analyses of diploid and related strains of yellow and white corn; of a number of commercial varieties of ordinary diploid corn, including examples of the more important kernel types, such as dent, flint, pop, and sweet corn; and of a limited number of diploid inbred lines and hybrids are shown in table 1. The total carotenoids in methyl alcohol, the active provitamin A fraction, containing carotene and cryptoxanthin, and the inactive zeaxanthin fraction are listed separately in the table. The sum of the active and inactive petroleum ether fractions does not in all cases equal the values obtained for the total carotenoids in methyl alcohol, presumably because of losses incurred during their saponification, transfer to petroleum ether, and final separation. Therefore, the values obtained from the original methyl alcohol extractions are considered to be a more reliable index of the relative amounts of total carotenoids present in the different kinds of corn that were analyzed.

The feeding experiments of Mangelsdorf and Fraps (*8*) demonstrated that the amount of vitamin A in corn meal was directly proportional to the number of dominant genes for yellow endosperm color present in the seed, the amount present being approximately in the ratio 3 : 2 : 1 for the *YYY*, *YYy*, and *Yyy* endosperm genotypes. White corn of the constitution *yyy* was found to have no vitamin A potency, a result which was in agreement with the earlier work of Steenbock and Boutwell (*14*) and Hauge and Trost (*2*). Results similar to those of Mangelsdorf and Fraps (*8*) were reported recently by Johnson and Miller (*3*) from spectrophotometric analyses.

TABLE 1.—*Carotenoid analyses of tetraploid and related diploid corn, diploid commercial varieties, inbred lines, and hybrids, in milligrams of pigment per gram of dry corn meal, each value being an average of four or more analyses*

[Standard errors are given for the total carotenoid values]

Corn sample	Total carotenoids in methyl alcohol	Sum of petroleum ether fraction	Beta-carotene and cryptoxanthin	Zeaxanthin
	Milligrams	*Milligrams*	*Milligrams*	*Milligrams*
Diploid Yellow	0.0261±0.0005	0.0243	0.0097	0.0145
Tetraploid Yellow	.0366± .0010	.0314	.0122	.0192
Tetraploid Yellow B	.0389± .0006			
Diploid White	.0067± .0003	.0049	.0018	.0031
Tetraploid White	.0054± .0001	.0042	.0017	.0025
Commercial varieties:				
Leaming Yellow	.0266± .0001	.0267	.0068	.0199
Cornell 11	.0282± .0002	.0201	.0056	.0145
Alvords White Cap Dent	.0073± .0002	.0051	.0019	.0032
Argentine Yellow Flint	.0479± .0001	.0422	.0160	.0262
Queen Golden pop	.0352± .0002	.0325	.0117	.0208
White Rice pop	.0034± .0001	.0027	.0008	.0019
Yankee Cheat flour	.0044± .0002	.0028	.0008	.0020
Golden Bantam sweet	.0071± .0004	.0126	.0031	.0095
Inbred lines:				
Dutton Flint	.0144± .0001	.0113	.0035	.0078
Bloody Butcher	.0210± .0002	.0131	.0039	.0092
Illinois A–2311	.0257± .0001	.0183	.0047	.0136
Webber Dent 2312	.0327± .0001	.0237	.0065	.0172
Luces Favorite W36–1	.0270± .0001	.0271	.0108	.0163
Onondaga White W36–2	.0057± .0001	.0038	.0016	.0022
Cornell 11 W36–3	.0639± .0006	.0651	.0313	.0338
Bloody Butcher W36–4	.0476± .0003	.0421	.0178	.0243
Hybrids:				
W36–2 × W36–1	.0109± .0001	.0103	.0043	.0060
W36–3 × W36–4	.0705± .0001	.0709	.0328	.0381
W29–3	.0326± .0004	.0290	.0119	.0171

In the present study a comparison was made between the carotenoid content of pure yellow diploid corn carrying the three dominant genes, *YYY*, for yellow, and a derived tetraploid with the doubled number of genes, *YYYYYY*, for yellow. Increasing the number of genes for yellow from three to six increased the total carotenoid content from 0.0261 mg. per gram of dry meal in the diploid to 0.0366 mg. in the tetraploid (table 1), an increase of 40 percent. This was the percentage increase for the Tetraploid Yellow strain compared with the related Diploid Yellow strain. There was approximately the same percentage increase for both the active provitamin A fraction containing beta-carotene and cryptoxanthin and the inactive zeaxanthin fraction. That is, the increase in carotenoid pigment carried with it a proportional increase in vitamin A potency. The intensity of endosperm color was approximately the same in the diploid and tetraploid strains.

Although the 40-percent increase in carotenoid content is attributed to chromosome doubling, it is recognized that the growing of the Tetraploid Yellow and Diploid Yellow strains for two generations in order to obtain sufficient material for the carotenoid analyses provided a limited opportunity for the segregation of genes affecting carotenoid content to take place independently within each of these strains. Such genotypic changes might either diminish or accentuate the differences in carotenoid content caused by chromosome doubling. However, the inbred lines from which these strains originated, namely, Illinois A, Webber Dent, and Luces Favorite, did not differ markedly

in carotenoid content (table 1), and the opportunity for gene segregation to occur was reduced to a minimum by practicing mass pollination rather than selfing or sib-crossing individual plants.

A second tetraploid yellow strain that was analyzed, Tetraploid Yellow B, had a carotenoid content of 0.0389 ± 0.0006 mg. This strain was a third-generation selection for yellow from a cross between Webber Dent 2312 and a white Spanish Flint inbred and was still segregating for endosperm color. The carotenoid content of this impure yellow tetraploid strain was appreciably higher than that of its diploid yellow parent, Webber Dent 2312, which was 0.0327 ± 0.0001 mg. Ordinarily the admixture of white with yellow strains reduces carotenoid content. (See table 1 and the analyses of Mangelsdorf and Fraps (*8*), Johnson and Miller (*3*), and others.) The relatively high carotenoid content of the Tetraploid Yellow B strain is attributed to the effect of chromosome doubling.

The diploid inbred lines from which the pure Diploid Yellow and Tetraploid Yellow strains originated, namely, Webber Dent 2312, Illinois A–2311, and Luces Favorite W36–1, were somewhat different with respect to both endosperm color and carotenoid content. The Webber Dent inbred had the deepest endosperm color and also the highest carotenoid content, but the Luces Favorite inbred, which had the least endosperm color of the three, had a somewhat higher carotenoid content than the deeper yellow Illinois A line. The mean value for the three inbred lines was 0.0292 mg. per gram of dry meal, as compared with 0.0261 mg. for the diploid strain produced by intercrossing these lines.

The total carotenoid content of the Tetraploid Yellow strain was not twice as great as that of the related Diploid Yellow strain, as might have been expected since other workers have shown that in ordinary diploid corn the carotenoid content is directly proportional to the number of dominant genes for yellow endosperm. The carotenoid content per unit volume [6] in the tetraploid was 40 percent greater than in the diploid, but in terms of gene number per cell there was more than a twofold increase, as will be seen from the section on Cell-Volume Relations.

The tetraploid white-endosperm strain and the diploid from which it was derived contained appreciable amounts of carotenoids including beta-carotene and cryptoxanthin, which are precursors of vitamin A (table 1). In these strains, doubling the number of chromosomes and genes caused a decrease of 19 percent in carotenoid content. These white strains, which are designated Tetraploid White and Diploid White, originated from a cross between an inbred line of an early flint corn commonly known as Spanish Flint and an inbred line of white Argentine Flint corn. Other white-endosperm types of diploid corn, including White Rice popcorn, Yankee Cheat flour corn, and Onondaga White Dent corn, had a lower carotenoid content than the Diploid White corn (table 1). Alvord White Cap Dent, a yellow dent corn with a white crown, was also very low in total carotenoids.

In all of the tetraploid strains investigated, the weight of the individual kernels was approximately 50 percent greater than that of the

[6] Since approximate measurements showed no significant difference in the density of the diploid and tetraploid kernels, the carotenoid content for unit weight, i. e., per gram of dry meal, is also the carotenoid content per unit volume.

related diploid. This percentage increase in kernel size is characteristic of most strains of tetraploid corn (*11*).

The commercial varieties and inbred lines of diploid yellow corn that were analyzed exhibited a wide range of values for total carotenoid content, as indicated in table 1. The highest value (0.0639 mg. per gram) was obtained from an inbred strain of Cornell 11, and the lowest values were from Dutton Flint (0.0144 mg.) and Golden Bantam sweet corn (0.0071 mg.). The inbred line of Cornell 11 had more than four times as much carotenoid as the Dutton Flint.

It was noted that the yellow appearance of the grain was not a reliable criterion of carotenoid content. For most of the commercial varieties there was a positive correlation between intensity of endosperm color and the amount of carotenoids present in the meal. Golden Bantam sweet corn was a conspicuous exception. This is a rich yellow sort, but it has the lowest carotenoid content of any of the yellow kinds that were compared. However, Argentine Yellow Flint, a very deep orange-yellow type, had a higher carotenoid value than the deep-yellow dent varieties Cornell 11 and Leaming. Among the inbred lines there was no consistent relation between endosperm color and total carotenoids. A deep-yellow Dutton Flint inbred had only about half as much total carotenoid as a medium-yellow inbred line of Luces Favorite, the values for the two lines being 0.0144 mg. and 0.0270 mg., respectively. One inbred line of Bloody Butcher had a rather low carotenoid content, while a second one had more than twice as much. The highest carotenoid value that was obtained for any inbred line or commercial variety was that of a Cornell 11 inbred, which had 0.0639 mg. of carotenoid per gram of dry meal, as compared with 0.0282 mg. for the commercial Cornell 11 variety from which it originated. However, the color of the endosperm was essentially the same in the inbred and in the parent variety. These results, demonstrating a lack of correspondence between carotenoid content and intensity of endosperm color, are in agreement with the results recently reported by Johnson and Miller (*3*, *4*).

The commercial double-cross hybrid W29–3 and its parent lines were analyzed to determine the influence of hybridization on carotenoid content. In the production of this hybrid,[7] the parent inbred lines were combined as follows: (W36–3 × W36–4) × (W36–2 × W36–1), the first-named line being the seed parent of each hybrid combination. Since the endosperm tissue in which the carotenoids of the kernel are localized is triploid and originates from the combination of two sets of chromosomes and genes from the seed parent with one set from the pollen parent, the influence of the seed parent should be twice as great as that of the pollen parent in determining the carotenoid content of the hybrid, provided the genes affecting carotenoid content act in the cumulative manner described by Mangelsdorf and Fraps (*8*) for the yellow endosperm genes. The value for the single cross (W36–2 × W36–1), involving a white-endosperm type, Onondaga White Dent, and a medium-yellow type, Luces Favorite, is in close agreement with the expected value, being nearer that of the seed parent than that of the pollen parent. The other single cross (W36–3 × W36–4), which involved two deep-yellow endosperm types,

[7] WIGGANS, R. G. Unpublished data.

Cornell 11 and Bloody Butcher (the yellow endosperm of this variety is obscured by the presence of red pericarp color), had more total carotenoids than the seed parent. This is of special interest since the seed parent, Cornell 11, had the highest value of any of the diploid types that were analyzed. The increase in the hybrid may have been due to the complementary action of additional genes for yellow contributed by the two parents. The plausibility of this explanation is strengthened by the recent discovery that yellow endosperm color in maize is dependent on the interaction of the dominant allelomorphs of two or more genes (*9*). The double-cross hybrid W29–3 had a somewhat lower carotenoid content than the expected value.

The extreme variation in the carotenoid content of inbred lines of yellow corn, including both the active provitamin A fraction and the inactive fraction, emphasizes the importance of carotenoid determination to evaluate the feeding quality of these strains and their hybrids. The carotenoid content of the Cornell 11 inbred line, which had the highest carotenoid value of the five yellow inbred lines that were analyzed, was more than four times as great as that of the Dutton Flint inbred, which had the lowest value of these five lines. Since endosperm color is not a reliable criterion of carotenoid content, except within very broad limits, chemical analyses or biological tests with animals are necessary to determine at all accurately the carotenoid value of a given sample of yellow corn. Furthermore, the results obtained from the analyses of strains of white corn indicate that some of them, as for example, Diploid White, possess sufficient provitamin A carotenoids to be detected by animal-assay experiments. However, the animal experiments that have been performed (*2*, *8*, *14*) have failed to demonstrate any significant vitamin A potency of white corn.

CELL-VOLUME RELATIONS

Chromosome doubling ordinarily results in an increase in cell size commensurate with the increase in nuclear volume caused by the presence in the nucleus of the double number of chromosomes. Autopolyploids of recent origin invariably have larger pollen grains and larger stomata than the forms with lower chromosome number from which they originated, but not so much is known about cell-volume relations in many other parts of the plant, as, for example, in the endosperm. Since the endosperm of the corn kernel is a simple tissue made up of relatively homogeneous cell components, it is favorable material for a study of the influence of chromosome doubling on cell volume.

At the present time very little is known about the influence of the changed conditions of cell volume and gene number per cell upon the action of specific genes following chromosome doubling. In diploid organisms certain genes exhibit differential quantitative action, while others do not, under conditions in which cell-volume relations remain relatively constant. In autotetraploids both cell size and gene number per cell are greater than in the parental diploid, thus creating changed conditions under which the action of the genes may differ from their known action in the diploid organisms. An investigation of cell-volume relations in diploid and tetraploid corn was undertaken to determine the extent to which the concentration of the genes for yellow

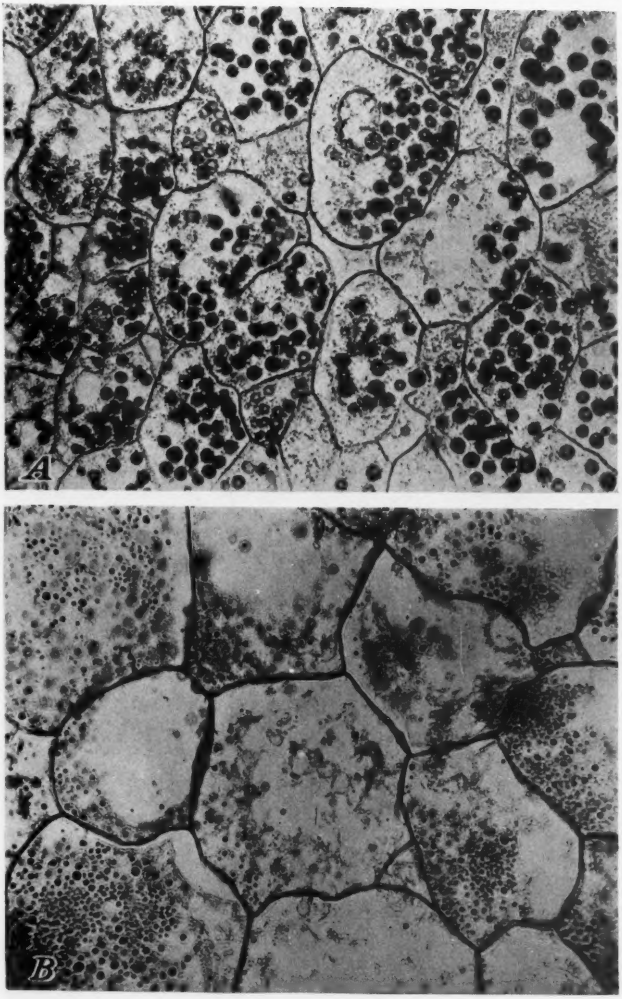
endosperm influenced the development of the character in question, i. e., the carotenoid content of the corn meal. This could be accomplished, since it was readily possible to determine cell volumes, and the degree of development of the character could be accurately determined by quantitative chemical and photometric analyses.

Since there is a twofold increase in the number of genes per cell in the tetraploid as compared with the diploid, the same concentration of the genes per unit volume will be maintained in the tetraploid if the volume of the cells is also doubled. If cell volume is not doubled, the concentration of the genes per unit volume will be increased in the tetraploid; if cell volume is more than doubled, there will be a corresponding decrease in gene concentration.

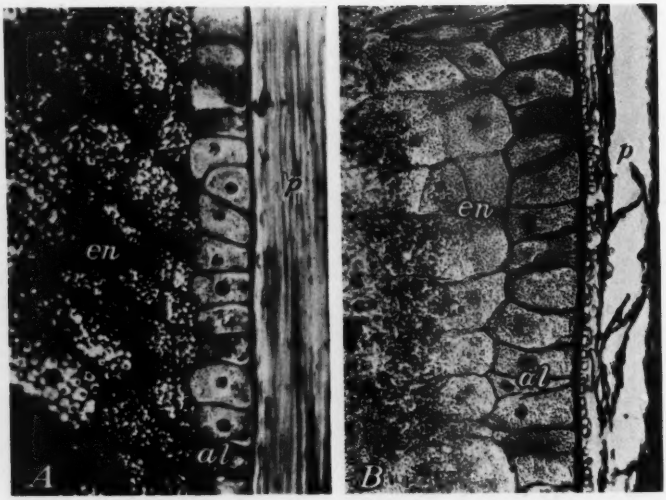
Computations of endosperm cell volume were made from the tissue in the central region of the endosperm and from the peripheral (aleurone) layer of cells in the midabgerminal region of mature kernels that in size and shape were representative of the diploid and tetraploid strains. In the middle region of the endosperm the cells were irregular in outline but isodiametric in longitudinal and cross sections. The relative volume of the cells in this region was computed by treating the cells as spheres whose areas were the areas of the cells in section view, areas being procured from planimeter measurements. The shape of the peripheral cells was essentially rectangular in both longitudinal and cross sections, their width when viewed in cross section of the kernel being somewhat less than their depth when viewed in longitudinal sections of the kernel. The volume of the aleurone cells was computed by multiplying together their three dimensions procured from the cross and longitudinal sections. Photomicrographs of representative regions of the endosperm from which cell measurements were taken are reproduced in plates 1 and 2.

For determining the mean cell volume in the two regions of the endosperm, five groups of six to eight contiguous cells were measured in each of four diploid and four tetraploid kernels selected as representative of the two strains. The values for cell volume obtained by this method of measuring all of the members of a given group of cells were somewhat lower than the true values, since the plane of section ordinarily was not median for all of the cells measured. Thus the maximum dimensions of all of the cells were not procured. The values are relative, not absolute, and provide an adequate basis only for a comparison of volume relations in the two kinds of corn. The computations of cell volume are given in table 2, including the means for each kernel and the means for all of the measurements from the two regions of the diploid and tetraploid kernels, together with their standard errors.

From the data presented in table 2 it is apparent that the endosperm cells in the tetraploid were very much larger than those of the diploid, the ratio of their volumes being essentially 3.6 : 1 in both the peripheral aleurone layer and in the central region. This is a much greater increase than has been reported in other studies of cell-volume relations following chromosome doubling. Nuclear volume in the same cells, as estimated from a limited number of measurements, was in the ratio of approximately 2.5 : 1, indicating that the ratio of chromosome numbers was 2 : 1 as expected in the endosperm of the diploid and tetraploid kernels that were examined.



Photomicrographs of endosperm tissue from the central region of the kernel in (*A*) diploid and (*B*) tetraploid corn taken at the same magnification. × 260.



Photomicrographs of the aleurone layer and adjacent tissues from kernels of (*A*) diploid and (*B*) tetraploid corn taken at the same magnification. Longitudinal sections from the midabgerminal region of the kernel: *al*, Aleurone; *en*, endosperm; p, pericarp. × 260.

TABLE 2.—*Comparisons of cell volume* [1] *in the endosperm of diploid and tetraploid corn*

Kernel No.	Central region		Aleurone layer	
	2*n*	4*n*	2*n*	4*n*
	Cubic millimeters	*Cubic millimeters*	*Cubic millimeters*	*Cubic millimeters*
1	175,478	645,068	5,155	18,380
	163,051	708,896	6,380	20,950
	153,710	549,181	6,730	15,700
	170,673	418,469	5,085	19,660
	163,051	755,536	4,655	17,420
Mean	165,193	615,430	5,601	18,422
2	157,960	448,943	4,245	21,160
	148,036	410,405	6,115	20,670
	145,132	349,585	4,490	22,600
	138,685	413,081	5,360	23,740
	180,374	571,698	5,730	31,280
Mean	154,037	438,742	5,188	23,890
3	119,560	469,084	6,360	18,080
	118,036	397,197	5,970	19,290
	141,118	386,833	7,120	20,820
	126,108	693,150	6,400	21,310
	188,455	575,773	6,365	23,800
Mean	138,655	504,407	6,443	20,660
4	143,285	610,112	4,870	21,240
	127,342	560,238	4,465	18,110
	152,216	490,720	6,020	17,260
	137,910	701,160	5,250	19,060
	128,782	530,272	5,830	20,750
Mean	137,907	578,500	5,287	19,284
Mean of all measurements	148,948 ±4,527	534,269 ±26,850	5,630 ±179	20,563 ±739
Ratio	1 : 3.58		1 : 3.65	

[1] Volumes were computed in cubic millimeters from measurements procured at a magnification of 530 diameters.

Although the individual cells of the tetraploid contained twice as many genes as did the cells of the diploid, the number of genes per unit volume was actually less in the endosperm of the tetraploid than in the diploid, owing to the pronounced increase in the volume of the cells of the tetraploid. Conversely, since the reduction in gene number per unit volume in the tetraploid was associated with a marked increase in carotenoid content per unit volume there was a very significant increase in the amount of carotenoid per gene elaborated by the tetraploid, and a still greater increase in the amount of carotenoid per cell. These proportional differences between the endosperm of the diploid and tetraploid strains are shown in table 3.

TABLE 3.—*Proportional differences between the endosperm of diploid and tetraploid strains of yellow corn*

Item	Diploid	Tetraploid	Item	Diploid	Tetraploid
Cell volume	1	3.6	Genes per unit volume	1	0.55
Carotenoid per unit volume	1	1.4	Carotenoid per cell	1	5
Genes per cell	1	2	Carotenoid per gene	1	2.5

The disproportionate increase in the size of the endosperm cells of the tetraploid, which were 3.7 times as large as those of the diploid, may have been due to their hexaploid constitution with which was associated a retarded nuclear and cell-division activity and a compensating increase in cell enlargment. Evidence in support of this view is furnished by octaploid corn plants, which invariably are much reduced in stature and have cells that are relatively very large and few in number. However, the kernels of the tetraploid were 50 percent larger than those of the diploid, and this increase was roughly proportional for the constituent parts of the kernel, including the endosperm, embryo, and pericarp. It is not known to what extent the increased size of the hexaploid endosperm was conditioned by the influence of other parts of the kernel that were tetraploid in chromosomal constitution.

The fact that in the tetraploid there was more than a twofold increase in the amount of carotenoid per gene could be interpreted to mean that a doubling of the number of genes per cell more than doubles the efficiency of each gene. But it might be argued that the endosperm is primarily a storage tissue and that the amount of carotenoid which it contains is conditioned by relationships existing in other tissues of the plant where carotenoids are being synthesized. For example, the leaf tissue contains appreciable amounts of the same carotenoids that are present in the endosperm and it is conceivable that the endosperm serves merely as a storage organ for a portion of the carotenoids synthesized by the leaves. However, it was shown by Johnson and Miller (*3*) that the amount of carotenoid in the leaf tissue of white- and yellow-endosperm sister lines was essentially the same, but the endosperm of the white lines contained very small amounts of carotenoid. Their work also substantiated the earlier results of Mangelsdorf and Fraps (*8*) to the effect that there is a positive correlation between the number of dominant genes for yellow and the amount of carotenoids in the endosperm of ordinary diploid corn. Thus it appears that the carotenoid content of the endosperm tissue is determined by the number of dominant genes for yellow endosperm present in the tissue rather than by conditions existing elsewhere in the plant; furthermore, from the results of the present investigation it may be concluded that the number of genes per cell unit is of more importance than the number of genes per unit volume in determining the amount of carotenoids present in the endosperm.

DISCUSSION

The results reported here have demonstrated a percentage increase following chromosome doubling in the carotenoids of yellow corn, and a percentage decrease in the carotenoids of white corn. Obviously, when there is a percentage increase in some of the substances in the corn kernel there must be a corresponding percentage decrease in other components, and vice versa. The effect of chromosome doubling on the relative amounts of other important constituents of the corn kernel, such as carbohydrates, proteins, and fat, was not determined in this investigation.

The percentage increase in the carotenoid content of the tetraploid yellow corn is interpreted as being due to a cumulative action of the dominant genes for yellow endosperm color. Since these dominant genes were not present in the white corn in an effective combination to

produce well-developed endosperm color, cumulative action was lacking in the tetraploid white corn and there was a resultant percentage decrease in carotenoids.

These results suggest that there are two categories of gene action in autotetraploids: (1) Cumulative gene action, which yields percentage increases and accounts for the distinctive traits of autotetraploids other than those that may be attributed directly to the presence of an increased number of chromosome sets, and (2) noncumulative gene action, which yields percentage decreases when the percentages of other constituents are increased. The significance of this classification can be understood better in the case of corn carotenoids if the quantity of pigment is expressed in amount per cell rather than in percentage. In the tetraploid yellow corn, owing to cumulative gene action, there was a fivefold increase in the amount of carotenoids per cell. In the tetraploid white corn, if it is assumed that the cell-volume relations in the diploid and tetraploid were the same as in the yellow corn, there was an increase in the amount of carotenoid per cell as a result of chromosome doubling; but this increase was very much less than in the yellow corn, owing to the absence of cumulative gene action. Further study of the differences that distinguish chromosome-doubled strains from their parent strains is needed, based on analyses of individual traits which may or may not be conditioned by cumulative gene action.

The relative importance of autotetraploids as horticultural and crop plants will be determined by the extent to which desirable traits are accentuated or produced by chromosome doubling without accentuating or producing undesirable traits. In terms of gene action, this means that chromosome-doubled strains of cultivated plants may have increased value if their desirable traits are controlled by genes that function in a cumulative manner to yield significant percentage increases like those reported here for the carotenoids of yellow corn. The fact that most of the more important crop plants are polyploids suggests that cumulative gene action has been an important determining factor in the evolution of cultivated plants.

SUMMARY AND CONCLUSIONS

Doubling the number of chromosomes in pure yellow corn caused a 40-percent increase in the content of carotenoid pigment.

The active provitamin A fraction of the carotenoids, including beta-carotene and cryptoxanthin, was increased in the tetraploid yellow corn approximately in proportion to the increase in total carotenoid pigment.

The volume of the endosperm cells of the tetraploid was approximately 3.6 times the volume of the endosperm cells of the diploid.

The increase in the cell volume and the carotenoid content of the endosperm in the tetraploid yellow corn resulted in a fivefold increase in the amount of carotenoid per cell.

The genes for yellow endosperm exerted a cumulative action following chromosome doubling. In the individual endosperm cells of the tetraploid the amount of carotenoid elaborated per gene was 2.5 times as great as in the individual cells of the diploid, even though there was a greater concentration of genes per unit volume in the diploid than in the tetraploid.

Doubling the number of chromosomes in white corn decreased the carotenoid content 19 percent. With respect to carotenoid content there was no cumulative gene action in the white corn.

The carotenoid content varied widely among different commercial varieties, inbred strains, and hybrids of ordinary diploid yellow corn. The inbred line with the highest carotenoid content had more than four times as much carotenoid as the line with the lowest carotenoid content.

The yellow appearance of the kernel was not a reliable criterion of carotenoid content.

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THE EFFECT OF ADDING BLACKSTRAP MOLASSES TO A LAMB-FATTENING RATION[1]

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INTRODUCTION

Considerable interest was manifested in the substitution of blackstrap molasses for a part, or all, of the grain in livestock rations during the drought years of 1934 and 1936. Ferrin [2] used molasses with corn and oats in fattening hogs and found that oats and molasses had a comparatively high value as compared with corn and molasses. Thompson and Hillier [3] experienced similar results in feeding swine on rations containing ample protein. Blizzard and Taylor [4] likewise found that oats and molasses made a more valuable ration for fattening steer calves than did corn and molasses when both rations were properly supplemented with cottonseed meal.

These results suggest that possibly oats and molasses supplement each other in the ration to a better advantage than corn and molasses. Forbes, Braman, et al. (*5*) have pointed out that the feeding value of a feed may vary widely depending upon the combination in which it is fed.

Patterson and Outwater (*12*) studied the influence of blackstrap molasses in a steer ration and reported that the molasses increased not only the palatability but also the digestibility of the ration. Lindsey and Smith (*9*) could not confirm these conclusions in a digestion trial with mature sheep and concluded that the digestion of all nutrients except fat was hindered when appreciable amounts of blackstrap molasses were included in the ration.

Snell (*16*) investigated the effect of molasses on the digestibility of steer rations. He used three yearling steers in each of two trials and found that various levels of molasses did not consistently alter the digestion of the nutrients in an ordinary steer ration consisting of corn, cottonseed meal, and grass hay.

A series of digestion trials was designed with lambs to find whether the addition of large amounts of molasses to an ordinary lamb-fattening ration would decrease the digestibility of the nutrients in the ration. The results of these trials are reported in this paper.

PROCEDURE

Four wether lambs were used in each of 3 separate trials, a total of 12 lambs being used in the study. All of the lambs were crossbred. One parent of each was a purebred Rambouillet and the other a purebred of either the Hampshire, Shropshire, or Southdown breeds. The lambs were all selected from the college flock and were thrifty, and hearty eaters. Each lamb of the 3 groups weighed approximately

[1] Received for publication July 26, 1939.

[2] FERRIN, F. E. CANE MOLASSES IN HOG RATIONS. Minn. Agr. Expt. Sta. Mimeographed Rpt. H–70. 1937.

[3] THOMPSON, C. P., and HILLIER, J. C. BLACKSTRAP MOLASSES AS A SUBSTITUTE FOR CORN AND OATS IN FATTENING RATIONS FOR HOGS. Okla. Agr. Expt. Sta. Mimeographed Cir. 8. 1938.

[4] Italic numbers in parentheses refer to Literature Cited, p. 71.

80 pounds at the start of the 3 trials, and all lambs gained during the experimental period.

The lambs were each fed test rations for a 10-day preliminary period and then placed in metabolism cages for a 10-day collection period. The cages were similar to those described by Forbes (*4*). The lambs ate readily after they became accustomed to the cages. The lambs usually gained slightly while in the cages on collection, but did not gain as much as they did in the pens during the preliminary periods.

The procedure used was the same as that previously reported by Briggs (*3*). Each lamb was fed the daily rations given in table 1. The feeds were carefully weighed on a scale sensitive to 0.1 gm. Each lamb was fed in an open bucket while in the preliminary pen, but in a specially constructed feeder while in the cage. The lambs were fed at approximately the same time each morning and evening and received one-half the daily ration at each feeding.

The corn and oats were fed whole while the alfalfa hay was ground in a hammer-mill and forced through a ¼-inch-mesh screen. The blackstrap molasses was weighed, diluted with water, and used to moisten the ration. The entire ration was thoroughly mixed before feeding.

Feces collections were made each morning at the same hour. Each day's collection was dried separately for 24 hours over an electric heater. The collection was then weighed and sealed. At the close of the 10-day collection period the dry feces were placed in a large container, thoroughly mixed, and representative samples were taken for chemical analysis.

TABLE 1.—*Quantitative composition (grams) of the daily rations used in digestion trials with lambs*

Component	Ration—			
	A	B	C	D
Corn (yellow)	460	230		
Oats			460	230
Alfalfa hay	454	454	454	454
Blackstrap molasses		230		230

The feeds given to lambs 1a, 2a, 3a, and 4a during the first digestion trial are shown in table 2. The trial was conducted during the interval from May 10 to August 26, 1937. The feeds used were representative of those produced during the dry growing season of 1936. Perhaps the extreme drought during the growing season caused the corn to be below normal in fat content. This analysis was checked and rechecked. In all other respects the feeds seemed to be normal in chemical composition. The market grades of the feeds used are also shown in table 2. The feeds used in the second trial were grown during the more normal reason of 1937. The composition and grade of these feeds are given in table 2 and they were consumed by lambs 1b, 2b, 3b, and 4b. Lambs 1c, 2c, 3c, and 4c were used in the third trial and were fed the feeds listed, with their chemical composition and grade, in table 2. These feeds were grown during the season of 1938. The market grades of the feeds used in these studies were secured through the courtesy of the Bureau of Agricultural Economics, United States Department of Agriculture.

RESULTS

Table 3 presents the apparent digestion coefficients secured in each trial. Each of the 12 lambs was fed consecutively on each of the 4 rations studied.

TABLE 2.—*Percentage composition and commercial grade of feeds used in three digestion trials with lambs*

Feed	Water	Protein	Fat	Crude fiber	Ash	Nitrogen-free extract	Grade
TRIAL 1, MAY 10 TO AUGUST 26, 1937							
Corn	14.01	9.90	1.91	2.21	1.55	70.42	[1] No. 5
Oats	9.66	11.71	3.74	10.24	3.92	60.73	No. 2
Alfalfa hay	10.31	15.14	.89	26.64	7.87	39.15	No. 2
Molasses	29.10	2.40			8.35	60.15	
TRIAL 2, FEBRUARY 25 TO JUNE 14, 1938							
Corn	13.89	9.89	3.19	2.14	1.29	69.60	No. 2
Oats	10.75	13.88	3.30	10.24	3.36	58.29	No. 2
Alfalfa hay	11.25	13.98	.89	27.57	7.73	38.58	No. 2
Molasses	31.72	2.00			9.40	56.88	
TRIAL 3, JANUARY 10 TO APRIL 14, 1939							
Corn	12.29	10.17	3.61	1.94	1.06	70.93	No. 2
Oats	9.49	12.38	4.46	14.38	3.62	55.67	No. 2
Alfalfa hay	5.99	14.75	2.95	25.50	6.93	43.88	No. 2
Molasses	35.40	1.62			9.22	53.76	

[1] Excess of shrunken kernels.

TABLE 3.—*Effect of blackstrap molasses on the apparent digestibility (percent) of lamb rations*

Lamb No.	Protein digestion coefficient for ration—				Fat digestion coefficient for ration—				Crude fiber digestion coefficient for ration—				Nitrogen-free extract digestion coefficient for ration—			
	A, corn, alfalfa	B, corn, alfalfa, molasses	C, oats, alfalfa	D, oats, alfalfa, molasses	A, corn, alfalfa	B, corn, alfalfa, molasses	C, oats, alfalfa	D, oats, alfalfa, molasses	A, corn, alfalfa	B, corn, alfalfa, molasses	C, oats, alfalfa	D, oats, alfalfa, molasses	A, corn, alfalfa	B, corn, alfalfa, molasses	C, oats, alfalfa	D, oats, alfalfa, molasses
1a	77.2	65.8	73.7	69.1	34.6	18.5	62.6	34.9	42.6	41.1	50.5	39.6	87.1	84.7	78.1	80.8
2a	73.0	73.4	74.7	63.5	35.4	21.9	57.0	47.5	52.5	29.6	50.4	39.1	85.1	88.3	80.2	79.9
3a	77.0	70.7	74.5	71.2	53.6	16.6	64.4	24.5	38.4	37.2	54.1	53.2	86.6	85.6	79.7	78.4
4a	78.9	72.0	77.8	67.7	55.8	20.0	76.0	37.9	51.1	41.9	46.1	40.0	86.2	86.0	81.8	81.1
Average	76.5	70.5	75.2	67.9	44.8	19.2	65.0	36.2	46.1	37.4	50.3	43.0	86.2	86.1	79.9	80.0
1b	66.0	63.0	73.1	67.1	49.0	27.0	64.4	45.0	46.0	45.9	52.1	45.7	83.6	82.2	80.0	77.0
2b	65.8	67.9	75.4	72.2	58.7	23.5	63.8	53.3	51.0	51.3	56.4	48.2	83.2	70.6	76.0	76.2
3b	67.5	60.4	75.4	68.7	48.7	45.6	62.9	37.1	46.4	43.5	54.3	48.7	84.3	81.9	78.3	74.5
4b	66.1	63.9	76.3	71.3	46.2	24.7	68.9	54.7	40.5	52.9	57.8	51.3	82.9	81.3	79.0	78.0
Average	66.3	63.8	75.0	69.8	50.6	30.2	65.0	47.5	46.0	48.4	55.1	48.5	83.5	79.0	78.3	76.4
1c	63.3	55.8	65.8	69.2	71.2	60.0	66.0	74.5	33.2	36.4	47.5	52.9	84.6	84.9	79.2	83.7
2c	64.2	61.4	69.3	63.1	68.3	60.7	80.5	65.7	30.5	25.2	45.1	43.7	85.5	83.8	76.4	83.6
3c	62.5	54.3	63.4	64.9	76.6	65.8	71.5	72.5	33.9	45.6	43.9	39.6	83.5	86.6	79.8	82.2
4c	62.8	68.0	66.2	62.1	66.3	51.5	81.2	68.1	38.6	37.3	45.5	42.5	86.5	85.9	76.2	80.7
Average	63.2	59.9	66.2	64.8	70.6	62.0	74.8	70.2	34.0	36.1	45.5	44.7	85.0	85.3	77.9	82.5
Average coefficient (3 trials)	68.7	64.7	72.1	67.5	55.4	37.1	68.3	51.3	42.1	40.7	50.3	45.4	84.9	83.5	78.7	79.7

DISCUSSION

The data presented in table 3 were analyzed by the method presented by Snedecor (*15*). The digestion coefficients of each nutrient were studied separately. Since the 12 lambs had been studied in 3 different periods, with different feeds, considerable difference existed among the coefficients of the separate periods. The difference between periods was highly significant in the case of protein, fat, and fiber coefficients, and it was therefore necessary to remove the variation between periods in the analysis of these data.

THE EFFECT OF BLACKSTRAP MOLASSES ON PROTEIN DIGESTION

The apparent digestion coefficients of the protein in the rations are shown in table 3. During the three trials, the replacement of one-half the corn ration for an equal weight of molasses lowered the coefficient of digestibility of protein in a corn-alfalfa hay ration 4 percent.

This difference did not prove to be significant when the effect of the periods was removed. In each separate trial, the digestibility of the ration had been lowered by molasses, but considerable difference existed in the digestion efficiency among the periods. This discrepancy was highly significant.

The digestibility of the protein in the oats-alfalfa ration did not vary much between periods. In two of the three trials, the average coefficient was considerably lower when blackstrap molasses was included in the ration while there was less decrease in the third trial. Lambs 1c and 3c gave slightly higher coefficients when the concentrates consisted of one-half blackstrap molasses. The average apparent digestion coefficient of protein was lowered 4.6 percent, and the difference was highly significant.

In a digestion study with dairy cows Williams (*17*) found that the addition of blackstrap molasses to the ration had a variable effect on digestion coefficients, but that in general the addition of the molasses lowered the digestion coefficient of protein. An analysis of the digestibility of protein of the basal rations showed no significant difference. However, most of the lambs digested a slightly higher percentage of the protein from the oats-alfalfa ration than they did from the corn-alfalfa ration. This finding is in agreement with that of Morrison (*10*) who gives oats as having a slightly higher digestion coefficient for protein than corn.

THE EFFECT OF BLACKSTRAP MOLASSES ON FAT DIGESTION

A substitution of blackstrap molasses for one-half of the grain in a lamb ration decidedly lowered the digestibility of the fat in the ration. The coefficient of apparent digestibility of fat of a corn-alfalfa hay ration averaged 55.4 percent for the 3 trials. When one-half of the corn was replaced by an equal weight of molasses the coefficient dropped to 37.1 percent or a difference of 18.3 percent. This decrease was highly significant. In each of the 12 lambs studied the addition of molasses decreased the digestibility of the fat in the ration. The fat in the rations proved to be more digestible in each succeeding trial. This may have been due to the efficiency of the lambs, but probably was the result of the varying character of the feeds.

In a similar manner the replacement of one-half the oats in an oats-alfalfa hay ration with an equal weight of molasses decreased the digestibility of the fat in the ration of 10 of the 12 lambs. Lambs 1c and 3c in the third trial digested the fat in the ration more efficiently when molasses was present. Notwithstanding, the average of the 12 lambs gave the oats-alfalfa ration a fat digestion coefficient of 68.3 percent as contrasted with 51.3 percent when molasses was added to the ration. This difference was highly significant.

Perkins and Monroe (*13*) noted a depression in the digestion coefficients of the nutrients in a dairy-cow ration when blackstrap molasses was included. The digestion coefficient of fat was lowered more than that of the other nutrients in the low protein rations which they used.

Morrison (*10*) gives the fat digestion coefficient of corn as slightly higher than that of oats. The corn used in this study had an average fat content of 2.90 percent, and the oats an average fat content of 3.83 percent. The alfalfa contained an average fat content of 1.58 percent. The lambs gave 12.9 percent higher fat digestion coefficients for the oats-alfalfa hay ration than for the corn-alfalfa hay ration. This difference proved to be highly significant. A difference in digestion coefficients of only 1.4 percent would be anticipated in favor of the oats-alfalfa hay ration from using the digestion coefficients of Morrison in calculating the expected efficiency with which lambs would handle each ration. Oats are higher in fat than corn; and if the fat is also more completely digested in certain lamb-ration combinations, it raises the total digestible nutrients or energy value of a ration above that anticipated. This may in part explain the higher regard that practical shepherds have for the fattening value of oats as compared with the total digestible nutrient value commonly assigned them.

THE EFFECT OF BLACKSTRAP MOLASSES ON CRUDE FIBER DIGESTION

Sheep and other herbivorae consume large amounts of crude fiber in their rations. It is therefore important to know what effect ration variations may have on the digestion of this nutrient. The addition of molasses slightly lowered the digestibility of the fiber in both the corn-alfalfa hay ration and in the oats-alfalfa hay ration. However, neither of these differences was significant.

The lambs digested the fiber in the oats-alfalfa hay ration to a greater extent than that in the corn-alfalfa hay ration. This difference proved to be highly significant. This is perhaps another factor in favor of oats as compared with corn as a feed for sheep. The effect of a feed upon the digestibility of a ration is no doubt more important than the way the single feed is digested when fed alone or in combination with certain other feeds.

THE EFFECT OF BLACKSTRAP MOLASSES ON NITROGEN-FREE-EXTRACT DIGESTION

The nature of the computation of the nitrogen-free-extract content of a feed or feces makes it rather an unsatisfactory value to study in digestion trials. It would seem logical that an increase in the sugar content of a ration would raise the nitrogen-free-extract digestion coefficients. A substitution of blackstrap molasses for one-half the grain in the rations of lambs had no material effect on the nitrogen-free-extract digestion of the ration.

In the third trial all four lambs showed a highly significant increase in the digestion of nitrogen-free extract when molasses was added to

an oats-alfalfa hay ration. This same addition caused no increase in the same ration in the other two trials, but showed a slight decrease in the second trial. In no case did the addition of molasses make a significant alteration of the nitrogen-free-extract digestion of a corn-alfalfa hay ration.

The lambs ate all four of the rations with relish and changed readily from the nonmolasses to the molasses rations and vice versa. The lambs were fed less than a full feed so that their appetites were always keen, and the feed was cleaned up regularly. During each experimental period the lambs gained steadily in weight and seemed to store some fat on their bodies.

Armsby (*1*) referred to a condition of "depression of digestibility" in rations that contain an excess of carbohydrates as compared with protein in a ration. Schneider and Ellenberger (*14*) encountered a depression of 4.0 ± 0.9 percent in the digestibility of fat in a dairy-cow ration low in protein as compared with one high in protein. The energy levels were approximately the same in both rations. Holdaway and coworkers (*6*, *7*) observed that unless adequate protein was present in a ration additions of apple pomace lowered the digestibility of the nutrients. Adding corn to a mixed-hay ration lowered the digestion coefficients of the protein, if a protein supplement was not used. The digestibility of the other nutrients was not materially changed by the addition of corn to the hay ration.

The nutritive ratios of the different rations used in this study varied, but all were as narrow as those suggested by Morrison (*10*) for the weight of lambs used. The computed nutritive ratio of the widest ration used was 1:7.24; Morrison's digestion coefficients were used in making these calculations. The lambs weighed an average of about 80 pounds each at the start of the experiments, and the average weight for the trial was approximately 90 pounds. It would not appear that the lambs used in this study were lacking in protein in view of modern feeding standards.

Nakamura (*11*) has shown that the administration of calcium lactate resulted in an increased excretion of fat and fatty acids in the feces of dogs. Likewise, Irwin, Weber, and Steenbock (*8*) have shown that large amounts of calcium and potassium chlorides will decrease the digestion of fat in the ration of the rat. Molasses is high in both calcium and potassium salts. It is therefore probable that the laxative effect of molasses experienced by the lambs while on the ration to which molasses was added lowered the digestion of fat and other nutrients.

It is possible that concentrated amounts of sugar in the rumen might interfere with normal functions in that organ. This consideration is probably not important as fiber digestion was not materially influenced, but the digestion of protein and fat nutrients, usually credited to the lower parts of the alimentary canal, was noticeably affected.

The large amounts of molasses fed in this trial are in excess of those that would likely be fed in common practice except in cases where molasses might be considerably cheaper than other concentrates.

SUMMARY

In a series of digestion trials the inclusion of large amounts of blackstrap molasses lowered the digestibility of certain nutrients in a lamb

ration. Four lambs were used in each of three separate digestion trials. The lambs were placed on each ration for a preliminary period of 10 days. They were then placed in a metabolism cage and the feces recovered for a like period.

The substitution of 230 gm. of molasses for the same amount of corn in a corn-alfalfa hay ration lowered the digestion coefficients of crude protein, but the decrease was not significant. The digestibility of fat was lowered 18.3 percent which was highly significant. The average crude-fiber and nitrogen-free-extract coefficients were lowered very slightly but not in significant amounts.

The replacement of 230 gm. of oats with molasses in an oats-alfalfa hay ration lowered the digestion of protein the highly significant amount of 4.6 percent. The digestibility of fat was decreased 17 percent, a highly significant amount. When the three trials are considered together, the changes in the digestibility of fiber and nitrogen-free-extract are not significant. In the third trial the addition of molasses to the oats-alfalfa hay ration did increase, to a highly significant extent, the digestibility of the nitrogen-free-extract of the ration.

The lambs used in the study digested the fat and fiber in an oats-alfalfa hay ration more completely than the smaller amounts of fat and fiber in a corn-alfalfa hay ration. The difference was highly significant.

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